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PHYSALIS FLORIDANA AS A LOCAL LESION TEST PLANT FOR POTATO VIRUS Y[1]

A. Frank Ross

In a previous report (8) the formation of local lesions on plants of *Physalis floridana* Rydb. following inoculation with potato virus Y (*Marmor upsilon* Holmes) and the use of such lesions in quantitative work were described. Darby, Larson and Walker (4) considered this species unsatisfactory because of inconsistency in the formation of lesions. Results obtained in this laboratory show that satisfactory quantitative work can be done on *P. floridana* if proper conditions are maintained. This plant also has proved useful for cross-protection tests (11). Since lesion formation is dependent upon the use of proper conditions, additional data on the conditions requisite for good lesion formation are included in this report, together with data on factors affecting number of lesions. Preliminary reports of some of this work have been published (9, 10).

MATERIALS AND METHODS.—Except where stated otherwise, all experiments were conducted in a greenhouse maintained at approximately 70° F. Plants were grown in 5-in. pots containing composted soil.

Preparation of inocula.—Unless otherwise stated, a strain of potato virus Y obtained from E. S. Schultz was used in all tests. It corresponds to isolate no. 19 of Darby, Larson and Walker (4) and is similar to or identical with the field strain most common in the northeastern part of the United States. The virus was maintained by serial transfer in Turkish tobacco (*Nicotiana tabacum* L.) or in *N. glutinosa* L. Inocula were taken from either of these 2 suscepts 2–3 weeks following inoculation. Leaves showing typical symptoms were ground in a meat chopper. Juice was extracted through cheesecloth, diluted with buffer, and used immediately.

Method of inoculation.—The gauze-pad method of inoculation was used in all cases. Unless otherwise stated, leaves of plants to be inoculated were dusted lightly with carborundum (400-mesh). Inoculated plants were rinsed with tap water as soon as possible after inoculation. Inoculations were made so that in a given experiment each inoculum was applied to each plant and at each leaf position the same number of times as each of the other inocula. In so far as it was practicable, the inoculation schemes were balanced with respect to other known variables. When 2 inocula were used, they were rubbed on opposite half-leaves. When more than 2 inocula were used, each was paired with each of the others in a balanced incomplete block arrangement.

All flower buds, unnecessary shoots, and all leaves except those to be inoculated were removed prior to inoculation.

Preparation of buffers.—Phosphate buffers were prepared by the mixing of solutions of monobasic and dibasic salts of phosphoric acid, each of the desired molarity, until the required pH was obtained. Unless specifically stated otherwise, the potassium salts of phosphoric acid were used. Borate buffers were prepared similarly from solutions of boric acid and sodium borate. In the preparation of all other buffers, a weighed quantity of the substance was dissolved in a volume of distilled water slightly less than that required to give the desired molarity. Sufficient N sodium hydroxide or N hydrochloric acid was added to bring the solution to the desired pH. The pH was again checked with a glass electrode following final adjustment to the required volume. All organic buffers were stored in a refrigerator and used within 2 days.

AGE OF PLANT.—Lesion formation was best on plants inoculated during early flowering. Younger plants not only had smaller and more tender leaves than older ones but when inoculated produced fewer and poorer lesions. There were fewer lesions per unit area and those were often indistinct and not easily distinguished. Satisfactory results were obtained with plants over about a 2-week period following the onset of flowering. As the leaves approached senescence, lesion formation became progressively less satisfactory.

At the age optimum for lesion formation plants of *P. floridana* will have from 12–18 leaves suitable for inoculation. The leaves are large, have a smooth upper surface, and are spaced so that they can be easily inoculated. On such plants inoculated uniformly with virus Y, lesion formation is greatest on the older leaves and decreases progressively acropetally. Use of immature leaves near the top should be avoided.

CONDITION OF PLANTS.—Only rapidly growing, vigorous plants were satisfactory. Since relatively large plants must be produced, 5-in. pots were used. Composted soil was supplemented with commercial fertilizer. Occasionally, supplementary applications of nitrogen were necessary a few weeks before flowering. On the other hand, *P. floridana* grew poorly in soil containing excessive amounts of soluble salts.

Adequate and uniform moisture was necessary. Occasionally, intumescences formed on the leaf veins and veinlets. This condition apparently was associated with fluctuating water supply and sometimes followed the root-pruning that occurred when plants were moved from one cinder bed to another. Oedema occurred infrequently in plants grown in pots placed in saucers. Plants from which all axillary shoots were removed when 2 in. or less in length were better formed and had more uniform leaves than did nonpruned plants.

LIGHT.—During winter months, supplementary light was found to be essential during the entire growing period. Good results were obtained with 1000-watt

[1] Accepted for publication September 16, 1952.

Mazda lamps, placed about 2 ft. above the plants at about 5-ft. intervals along the bench. They were used every evening until 10 P. M. and on all cloudy days. Exposure of plants to short day-lengths for prolonged intervals any time after emergence resulted in poorly formed plants with many small leaves.

Plants placed in shade or in the dark for a few days just prior to inoculation were more susceptible than those exposed to normal greenhouse light or to supplementary light. The phenomenon is similar to that observed by Bawden and Roberts (1, 2) in the case of several virus-suscept combinations. In one experiment, 2 plants were placed so that a branch of each was enclosed in a specially constructed box lined with black paper. Two days later the boxes were removed and all leaves uniformly inoculated with diluted infectious juice. On the 13 darkened leaves 461 lesions developed while 318 developed on the 13 leaves exposed to normal light. In a similar experiment, employing 6 plants and a 4-day dark period, 2843 lesions developed on the 30 leaves that had been darkened and 1856 developed on the 30 that had been exposed to normal light.

Variations in the amount of light supplied during the post-inoculation period had little or no effect on lesion formation.

Temperature.—The temperature at which plants of *P. floridana* were grown prior to inoculation had no great effect on subsequent lesion formation. At uniform post-inoculation temperatures, plants which had been grown at 80° F. produced about 20 per cent more lesions than similarly inoculated plants which had been grown at 65° F. However, the lesions on plants grown at 65° were more distinct and more easily counted than were those on plants grown at 80°. Physalis plants grown at about 70° F. have proved satisfactory for routine use.

Post-inoculation air temperatures were found to be very important. Lesions on plants held at temperatures between 65° F. and 70° F. were invariably well formed and easily distinguishable. This temperature range also is optimal with respect to number of lesions produced. At 75° F., about 30 per cent fewer lesions were produced than at 65° and they were small, indistinct, and difficult to count. At 80° F., no lesions formed although infection occurs at this temperature (11).

Lesion formation is satisfactory under conditions where the temperature fluctuates, provided temperatures of 80° F. or above are not maintained for extended periods. In the greenhouses at Ithaca, lesion formation has been poor or non-existent only during July and August. Although day-time temperatures often exceed 80° during certain other months, cooler temperatures apparently prevailed for periods sufficiently long to permit fair lesion formation.

Use of carborundum.—The use of carborundum increased lesion formation approximately 7-fold. In a typical experiment, the left halves of 36 leaves were inoculated with diluted infectious juice. The leaves were dusted immediately with 400-mesh carborundum and the remaining half-leaves inoculated. The leaves were rinsed immediately following completion of the second inoculation. The process was repeated on another set of 36 half-leaves except that the right halves were inoculated before application of carborundum and the left halves afterward. Only 104 lesions developed on the carborundum-free leaf-halves while 723 developed on the dusted ones. Subsequent experiments showed that while the interval between the inoculation and the rinse in the above test was greater for the nondusted leaves than for the dusted ones this fact could not account for the observed differences in lesion count. It will be shown later that satisfactory quantitative results can be obtained on leaves dusted with carborundum.

Depletion of inoculum and extent of rubbing.—With some viruses, the frequency with which the inoculum is renewed on the inoculating-pad markedly affects the number of lesions (5), while with others it has little or no effect (15). Lesion count might be expected to increase as the number of strokes used in rubbing a leaf is increased but in some cases excessive rubbing decreases the number of lesions (5). Consequently, it was of interest to determine the effect of these 2 variables on Y-lesion formation. The possibility that the two are interrelated also was investigated.

In a preliminary test on depletion, a single inoculum (infectious juice diluted 1-50 with 0.1M phosphate buffer) was uniformly rubbed (6 strokes per half-leaf) over the surfaces of 300 half-leaves on 10 plants. Ten half-leaves were inoculated before renewal of inoculum on the pad. The lesion totals for successively inoculated leaves were 10660, 10174, 8988, 8010, 7493, 6732, 5998, 5251, 4859, and 3974, respectively. In a similar experiment juice diluted 1-100 with 0.1M glycylglycine (pH 7) was similarly rubbed over 200 half-leaves on 10 plants. The lesion totals for successively inoculated leaves were 3470, 2935, 2624, 2373, 2048, 1605, 1428, 1094, 1044, and 726, respectively.

In a preliminary test on the extent of rubbing, the half-leaf technique was used for a direct comparison of 3 strokes with 6, 6 with 9, and 9 with 12. The inoculum was juice diluted 1-200 with 0.01M phosphate buffer (pH 7). Twenty-four leaves were used for each comparison and the inoculum was renewed on the pad after each third half-leaf. Three strokes resulted in 133 lesions as compared to 149 with 6. Six strokes gave 139 in comparison to 91 with 9. Nine strokes gave 75 as compared to 39 with 12.

That the depletion effect is a function of the extent of rubbing was indicated by 2 experiments in which the number of strokes was varied and the lesion counts tabulated according to whether the leaf was the first, second, or third one rubbed following renewal (Table 1). Results in general agree with those of the preliminary tests in that maximum lesion count was obtained with 6 strokes and in that there was a progressive decrease in number of lesions on successively inoculated leaves. In addition, there was a marked tendency for depletion to be more rapid as the number

TABLE 1.—*Lesions produced on successively inoculated Physalis floridana leaves with the number of strokes per half-leaf varied*

Test	No. of Strokes	First half-leaf	Second half-leaf	Third half-leaf	Stroke totals
1[a]	3	1267	1328	1048	3643
	6	1557	1477	1115	4149
	9	1584	1261	1064	3899
	12	1327	942	653	2922
Leaf Totals		5735	5008	3870	
2[b]	3	1098	970	973	3031
	6	1418	1311	1271	4000
	9	1454	1327	1162	3943
	12	1508	1267	1049	3824
Leaf Totals		5478	4875	4445	

[a] Each subtotal is the number of lesions on 12 half-leaves. L.S.D. (0.05 level) is 341 for subtotals, 591 for stroke totals, and 682 for leaf totals. Uniform inoculum used was prepared by diluting infectious juice 1-100 with 0.01M phosphate buffer (pH 7).

[b] Each subtotal is the number of lesions on 12 half-leaves. L.S.D. (0.05 level) is 273 for subtotals, 473 for stroke totals, and 574 for leaf totals. Uniform inoculum was prepared by diluting infectious juice 1-100 with 0.1M glyclyglycine buffer (pH 7).

of strokes was increased.

Data in Table 1 suggest that depletion and the extent of rubbing had a more pronounced effect on lesion formation when phosphate buffer was used as diluent than when glycylglycine buffer was used. That these differences were due to some other variable and not to the buffer was shown by other experiments. It was common experience for the rapidity of the depletion of inoculum and the depression of lesion formation by excessive rubbing to vary from experiment to experiment; however, within a given experiment, use of the 2 buffers always gave comparable results.

Six strokes per half-leaf with renewal of inoculum after each third half-leaf were adopted as standard procedure. More frequent renewal was not found practical because of the greater time required and because the excess inoculum deposited would often drop from upper leaves to lower ones.

RINSING.—Preliminary tests indicated that the standard practice of rinsing immediately following inoculation tended to increase lesion-counts in some cases but not in all. Direct comparisons of rinsed with non-rinsed leaves usually resulted in higher counts on the rinsed leaves. Since the suscept under investigation has several leaves and a single group of plants can be used for a large number of inocula, inoculation schemes can become quite complex and necessitate considerable time for inoculation. Hence it appeared desirable to investigate the time relationships of the rinsing effect.

Infectious juice was diluted with buffer and the diluted juice divided into 4 equal portions. One portion was used for the inoculation of ¼ of the half-leaves on 6 plants; each with 6 leaves. Four to 6 minutes later another set of half-leaves was rubbed with another portion of the inoculum. The other 2 sets were subsequently inoculated at intervals of 2–4 minutes. As soon as the final half-leaf on a plant was inoculated, an assistant rinsed the plant with tap water. The same procedure was repeated on a second lot of 6 plants. The whole procedure was timed accurately so that the average time between the inoculation and the rinse for each set of half-leaves could be calculated.

When 0.1M borate buffer (pH 8) was used as diluent, lesions appeared only on those leaves rinsed within a minute. All other leaves were severely injured by the buffer. When 0.01M phosphate buffer (pH 7) was the diluent, there often was a marked progressive decrease in number of lesions as the interval between inoculation and the rinse was lengthened. In the experiments reported (Table 2) and in others where rinsing had an effect, there was a rather rapid decrease with intervals up to about 3–4 minutes. During subsequent intervals the decrease was less marked and was essentially linear. In a number of tests with this buffer, the effect of rinsing was not always as pronounced as in the experiments reported and in a few cases there was no effect. Apparently, other variables are important in determining the magnitude of the rinsing effect.

Results of tests in which 0.1M glycylglycine buffer (pH 7) was used as diluent were similar to those where 0.01M phosphate buffer was used, except that in general the rinsing effect appeared to be less marked.

Attempts to identify the variable or variables causing fluctuations in the magnitude of the rinsing effect were not successful. Direct comparison of plants inoculated in bright sunlight and in shade gave no consistent results. No correlation could be detected between environmental conditions or the condition of the

TABLE 2.—*Number of lesions produced on Physalis floridana inoculated with uniform inoculum but with interval between inoculation and the rinse varied*

Experiment[a]	Interval[b]	Lesions[c]
	Min.	
1	0.5	4814
	4.0	3796
	8.0	3463
	12.0	3166
L.S.D. (0.05)		675
L.S.D. (0.01)		893
2	0.2	3343
	2.0	2992
	5.5	2523
	10.5	2116
L.S.D. (0.05)		593
L.S.D. (0.01)		784

[a] Infectious juice diluted with 0.01M phosphate buffer (pH 7). The dilution was 1-50 in experiment 1 and 1-100 in experiment 2.

[b] Average time between inoculation and rinse.

[c] Number of 36 half-leaves. A balanced incomplete block arrangement of inoculated half-leaves on 12 plants, each with 6 leaves, was used.

plants at the time of inoculation and the magnitude of the rinsing effect.

Since rinsing may have a marked effect on lesion-count and the importance of this effect is unpredictable, it is important that inoculation schemes be designed so that in a given test the average time between inoculation and the rinse is the same for each inoculum.

BUFFERS.—Since 0.1M phosphate buffer at pH 7 is used widely in virus work, this buffer was used in the early experiments. It soon was discovered, however, that the use of certain other buffers resulted in greatly increased lesion counts. Screening tests were made in which were tested, for the most part, only those substances that are good buffers near pH 7. In general, inoculations were made in a manner that permitted removal of excess inoculum by rinsing within a minute following inoculation. Only the simplest of inoculation schemes, that involving 2 inocula, was used. Buffers adjusted to pH 8 were prepared from maleic acid, boric acid and sodium borate, arginine-HCl, asparagin, lysine-HCl, and glycylglycine. Buffers adjusted to pH 7 were prepared from maleic acid, histidine-HCl, glycylglycine, and the sodium and potassium salts of phosphoric acid. Various buffer concentrations between 0.01M and 0.1M were tested. In these screening tests on *P. floridana*, highest lesion counts resulted from the use of 0.1M borate (pH 8), followed in order by 0.1M glycylglycine (pH 7), 0.05M histidine (pH 7) and 0.01M phosphate (pH 7). In tests with a given type of buffer, the number of lesions varied with buffer concentrations and with pH. Consequently, the 3 most promising substances were used for further tests. Since results obtained with sodium phosphate buffer were similar to those with potassium phosphate buffer, only the latter was used in subsequent tests.

Buffer concentration.—Borate, glycylglycine, and phosphate buffers were prepared, each at 5 different concentrations varying from 0.001M to 0.1M. In tests involving a single buffering substance, infectious juice was diluted 1-100 with each of the 5 buffers differing only in concentrations. A 2-solution inoculation scheme was used for the direct comparison of each sample with the one diluted with the next most concentrated buffer. Inoculated leaves were rinsed within a minute following inoculation.

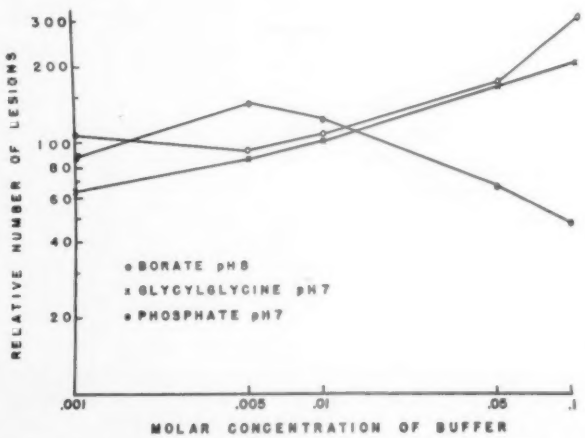

FIG. 1. Relative number of lesions on plants of *Physalis floridana* inoculated with infectious juice diluted 1-100 with buffers differing in concentration.

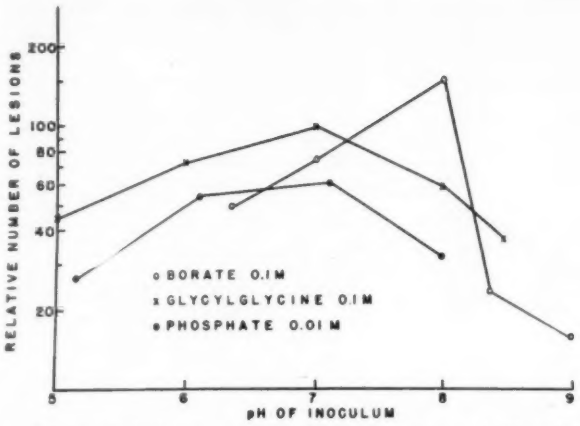

FIG. 2. Relative number of lesions on plants of *Physalis floridana* inoculated with infecious juice diluted 1-100 with buffers adjusted to different pH values.

In the phosphate series, maximum lesion formation was obtained where 0.005 M phosphate was used as diluent (Fig. 1). Concentrations approaching 0.1M had a very definite inhibitory effect. On the other hand, no maximum was reached in the glycylglycine series. Within the limits tested, the greater the buffer concentrations, the greater was the lesion count. The same was true in the borate series except that use of 0.001M borate resulted in slightly more lesions than did use of 0.005M borate. Results with the higher concentrations of borate were rather striking. An increase in concentration from 0.05M to 0.1M caused a sharp increase in lesion formation. This effect has been observed repeatedly and is of particular interest, for phytotoxicity also increases sharply over the same range.

While the curves in Fig. 1 were constructed from data from 3 independent experiments, they were actually located relative to each other with the aid of data from another experiment. The 3 buffers, each at 0.01M concentrations were compared as diluents by the method previously described. The relative lesion counts from this experiment then were located on the graph and each curve drawn through the appropriate points. Thus each point in Fig. 1 is more or less relative to each other point.

pH of buffers.—Series of 0.1M borate, 0.1M glycylglycine and 0.01M phosphate buffers, each varying in pH, were prepared and compared as diluents. Infectious juice was diluted 1-100 with each of the buffers and used for inoculation of plants of *P. floridana*. In the borate series, a 2-solution scheme was used so that leaves could be rinsed soon after inoculation. Since no evidence was obtained of any marked phy-

TABLE 3.—*Number of lesions produced on plants inoculated with infectious juice diluted with different buffers*

Diluent	Physalis floridana[a]		41965[b]	
	No. half-leaves	No. lesions[c]	No. half-leaves	No. lesions[c]
Distilled water	24	1037		
0.01M phosphate pH 7	24	1551**		
0.01M phosphate pH 7	50	1082	28	501
0.1M glycylglycine pH 7	50	1914**	28	528
0.1M glycylglycine pH 7	50	1151	40	606
0.1M borate pH 8	50	2216**	40	1122**
0.1M borate pH 8	18	297**	36	659**
0.01M phosphate pH 7	18	78	36	380

[a] Juice from tobacco or *Nicotiana glutinosa* plants infected with virus Y was diluted 1-100 with the indicated diluent.
[b] U.S.D.A. potato seedling 41956. Infectious juice diluted 1-25 with indicated buffer.
[c] The figures in these columns represent the number of lesions formed on the indicated number of half-leaves when the samples paired vertically were rubbed on opposite half-leaves. The double asterisk means that analysis by Student's t test indicates that the probability is less than 0.01 that the observed difference would occur in a homogeneous population.

totoxic effects of glycylglycine or phosphate, multiple-solution schemes, similar to that described in a later section, were used in the series involving these 2 buffers.

Results with phosphate and glycylglycine were similar in that optimum lesion formation resulted from the use of buffers at about pH 7 (Fig. 2). Results in the borate series were strikingly different, for in this case maximum lesion formation resulted from the use of buffer adjusted to pH 8. As the pH of the borate buffer was increased, phytotoxicity also increased. Hence, as in the borate series in Fig. 1, those borate solutions that are the least phytotoxic were not necessarily the ones that permitted maximum lesion development.

The technique described in the previous section was used for drawing Fig. 2 so that any one point can be compared with any other point.

Comparison of diluents.—Data obtained in the preceding experiments indicate that on *P. floridana*, use of 0.1M borate buffer (pH 8) results in more lesions than that of 0.1M glycylglycine buffer (pH 7) while fewest lesions are obtained from the use of 0.01M phosphate (pH 7). This conclusion was substantiated in further tests where each buffer was compared as diluent with each other buffer (Table 3). When 0.1M borate (pH 8) was used as diluent, approximately twice as many lesions developed as when 0.1M glycylglycine (pH 7) was used. Use of the latter buffer resulted in about twice as many lesions as did use of 0.01M phosphate (pH 7). While the phosphate buffer was the poorest used, it proved to be considerably better than distilled water.

Results with borate buffers were similar when other suscept were used as test plants, but the glycylglycine buffer was no better than the phosphate buffer. When USDA potato seedling 41956 (*Solanum tuberosum* L.) was the test plant, use of borate resulted in twice as many lesions as did use of any other buffer, while use of glycylglycine resulted in about the same number of lesions as did use of phosphate (Table 3). In a comparative test on 6 plants of *Lycium halimifolium* Mill. 36 leaves were rubbed with infectious juice diluted 1-50 with the borate buffer, another 36 with juice similarly diluted with glycylglycine buffer, and 36 with juice similarly diluted with the phosphate buffer. Lesion totals were 792, 392, and 380, respectively. In a similar test of 3 plants of *L. chinense* Mill., each with 15 leaves, the lesion totals were 156, 36, and 44 respectively. Glycylglycine buffer was not used as diluent in tests on *Chenopodium urbicum* L., but in a test with 0.1M borate (pH 8) and 0.01M phosphate (pH 7), 30 leaves inoculated with juice diluted 1-30 with the borate buffer had 2553 lesions while 30 leaves on the same plants inoculated with juice diluted similarmly with the phosphate buffer had 1000 lesions.

The data show that borate at pH 8 has a beneficial effect on lesion formation regardless of the test plant used. Only on *P. floridana*, on the other hand, is glycylglycine a better diluent than phosphate. Borate buffers are not suitable for routine work because of their excessive phytotoxicity. Consequently, the glycylglycine buffer is recommended for tests on *P. floridana* and either the glycylglycine or the phosphate buffer for tests on other suscepts.

STRAINS OF VIRUS Y.—Many isolates of potato virus Y from naturally infected potatoes have been tested on *P. floridana* and all produced local lesions. Two strains supplied by H. H. McKinney and described as "milder" than the type strain were tested as were a "mild" and "severe" strain supplied by R. H. Larson. All produced local lesions. While there were minor differences in the appearance of the lesions and in the rate of lesion development, results indicate that *P. floridana* can be used for a variety of strains differing in their effects on other suscepts.

OTHER VIRUSES.—*P. floridana* is susceptible to several viruses, some of which interfere with Y-lesion formation. Tobacco mosaic virus (*Marmor tabaci* Holmes) causes large necrotic blotches and leaf-drop. Infected plants are useless for assay work. Presence of the virus in Y-inocula results in loss of all leaves that become infected with tobacco mosaic virus. Plants infected with cucumber mosaic virus (*Marmor cucumeris* Holmes) react to inoculation with virus Y by the formation of local lesions (11) but the leaves on infected plants may be small and malformed. The effect of the presence of this virus in Y-inocula has not been determined.

Since virus Y is often found together with potato virus X (*Annulus dubius Holmes*) in natural infections, more extensive tests were made. *P. floridana* was susceptible to all tested strains of virus X but the symptoms developed and the effect on Y-lesion de-

velopment depended upon the particular strain used. The more severe strains, such as the ringspot strain, produced local lesions very similar to those caused by virus Y. Strains of virus X that are masked in tobacco and in *Datura stramonium* L. also are masked in *P. floridana*. Presence of such strains in Y-inocula does not prevent Y-lesion formation but does retard the rate of lesion development. This is illustrated by data from an experiment in which an inoculum containing virus Y alone was compared on opposite half-leaves with an inoculum containing the same amount of virus Y to which was added a masked strain of virus X. Lesions were counted 12, 14 and 16 days following inoculation. On these dates, the half-leaves inoculated with the mixture had 29, 57 and 93 per cent, respectively, as many Y-lesions as did those inoculated with virus Y alone. In routine experiments, it is not practicable to delay counting until the 16th day for leaf abscission often occurs about then. Also, if the presence of virus X is unsuspected, counts on the 12th day, usually the best day for counting, would lead to erroneous conclusions.

Inocula containing virus X can be assayed for relative amounts of virus Y if plants of *P. floridana* systemically infected with a masked strain of virus X are used. Plants were inoculated while quite young with masked X. Such plants were symptomless, and grew as well as did healthy plants. When inoculated with virus Y, they reacted just as did plants that were healthy when inoculated. Presence of virus X in the inoculum did not affect either the rate of lesion formation nor the number of lesions. This was true regardless of the strain of virus X used. Such "protected" or "immunized" plants have been used routinely in this laboratory for the assay of virus Y in the presence of virus X.

USE OF PHYSALIS FLORIDANA IN QUANTITATIVE WORK.—Since plants with 16 to 18 good-sized leaves can be used, *P. floridana* is well suited for inoculation schemes involving several inocula. The 4-inocula scheme shown in Table 4 has been used regularly and illustrates the degree of balance that can be obtained.

The growth habits of *P. floridana* are such that plants can be trimmed to 2 main branches. At a given leaf position, the leaves on both branches are very nearly the same size and age and react similarly when inoculated with virus Y. Each of 6 or 12 plants are trimmed to 6 pairs of matched leaves. Each inoculum is paired on opposite half-leaves with each other inoculum once on each branch and once at each leaf position (in each set of 3 plants). The subscripts denote whether the leaf is the first, second, or third one inoculated following renewal of inoculum on the pad. On a given plant, the 2 matched leaves have the same subscript. A given subscript appears but once at a given leaf position in each set of 3 plants. If 6 or 12 plants are used, each inoculum appears on the right as often as it appears on the left on each plant and at each leaf position. If 4 sets of 3 plants each are inoculated in the orders indicated in Table 4, the average time between inoculation and the rinse will be the same for each inoculum. Where a high degree of accuracy is required, 12 plants should be used. In tests requiring less accuracy, 6 plants can be used without introducing excessive error due to differences among the inocula with respect to the interval between inoculation and the rinse. If information is desired on the effect of variables other than the different inocula, the data may be analyzed by the analysis of variance method. If the significance of the differences among the inocula is the only information needed, the data may be analyzed by the balanced incomplete block method (3).

TABLE 4.—*A balanced inoculation scheme for use on Physalis floridana*[a]

	Plant 1		Plant 2		Plant 3	
Leaf	Branch I	Branch II	Branch I	Branch II	Branch I	Branch II
6	A B 3	C D 3	C A 1	D B 1	B C 2	A D 2
5	D C 3	B A 3	B D 1	A C 1	D A 2	C B 2
4	B D 2	A C 2	D A 3	C B 3	A B 1	C D 1
3	B C 1	A D 1	A B 2	C D 2	C A 3	D B 3
2	C A 2	D B 2	B C 3	A D 3	D C 1	B A 1
1	D A 1	C B 1	D C 2	B A 2	B D 3	A C 3

Plant numbers	Scheme	Order of inoculation
1-3	As shown	A B C D
4-6	Leaf-halves reversed	D C B A
7-9	As shown	C A D B
10-12	As for plants 4-6	B D A C

[a] Inocula are represented by letters and the sequence of a given pair of letters indicates which inoculum goes on the left half-leaf and which on the right. Subscripts denote whether the leaf is the first, second, or third one inoculated with a given inoculum after renewal of inoculum on the inoculating-pad. Each set of 3 plants should be rinsed immediately following completion of the inoculation.

Inoculation is facilitated by the use of bi-colored marking tags hung on the petiole of each leaf. Colors are used to indicate the inocula and 1–3 notches in the margin of the tag to indicate whether a leaf is the first, second, or third one inoculated following renewal.

Dilution curve.—Multiple-solution schemes such as that described were used for determining dilution curves of juice from infected plants. In almost every case, curves similar to that shown (Fig. 3) were obtained when buffers were used as diluent and the test plant was *P. floridana*. When distilled water was

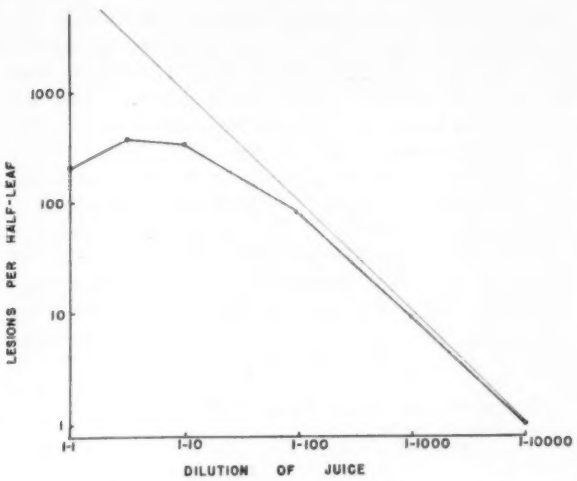

FIG. 3. Number of lesions obtained on plants of *Physalis floridana* with different dilutions of juice from plants of *Nicotiana glutinosa* infected with virus Y. The juice was diluted with 0.1M glycylglycine buffer (pH 7). Each inoculum was applied to 24 half-leaves. A balanced incomplete block arrangement of inoculated leaves was used. The straight line has a slope of unity and represented actual dilution of the virus.

used as diluent, the curve differed in that use of juice diluted 1-$\sqrt{10}$ did not result in a greater number of lesions than did use of undiluted juice. It would appear, therefore, that the increase in lesion count with dilution in the upper part of Fig. 3 is due to the beneficial effect of the buffer used as diluent.

Detection of differences in virus concentration.—Inocula differing in virus Y concentration by 20 per cent consistently gave differences in lesion counts that were statistically significant, provided each inoculum was applied to at least 36 half-leaves. When 72 half-leaves were used, inocula differing in virus concentration by only 10 per cent usually gave significantly different lesion counts. Thus the sensitivity of the local lesion method when this virus-suscept combination is used appears to be of the same order of magnitude as it is with most other virus-suscept combinations that have been tested.

Potency assay.—The 2-dilution potency assay method of Spencer and Price (13) was applied to solutions differing in virus Y content by known amounts. The 4-inocula scheme shown in Table 4 was used in some cases and in others various modifications were used that made use of different numbers of leaves. Potency of the "unknown" was calculated by the formula suggested by Sherwood (12). In a given test, an "unknown" of known potency relative to the standard was prepared by dilution of the juice used as standard or *vice versa*.

In 8 of the 9 tests, the estimated potency of the "unknown" was within 10 per cent of the actual potency (Table 5). In the lone exception, the 2 differed by about 12 per cent. Thus, it appears that relative concentrations of virus Y can be determined with a degree of accuracy comparable to that obtained in the assay of other viruses (6, 7, 13).

TABLE 5.—*Estimation of virus Y concentration by comparison of 2 dilutions on Physalis floridana*

No. of half-leaves[a]	Dilutions used[b]		Potency of "unknown"[c]	
	Standard	Log interval	Actual	Estimated
36	1-250	0.5	2.50	2.59
64	1-300	1.0	1.67	1.80
36	1-150	0.5	1.43	1.62
36	1-100	0.5	1.00	0.98
36	1-100	0.5	1.00	0.98
36	1-100	0.5	0.70	0.69
72	1-100	0.5	0.70	0.67
36	1-100	0.5	0.40	0.39
72	1-100	0.5	0.40	0.39

[a] Number of half-leaves inoculated with each dilution.
[b] In a given test, juice from either infected tobacco or *Nicotiana glutinosa* plants was used for the preparation of both standard and unknown. A standard was prepared by diluting juice as indicated in column 2 and the "unknown" by diluting juice so that its virus content differed from that of the standard by the ratio indicated in column 4. An additional sample of each differing in dilution from the above by the log interval indicated in column 3 also was prepared.
[c] Ratio of virus concentration of "unknown" to that of the standard. Estimated values were calculated by the formula given by Sherwood (12).

DISCUSSION.—Yarwood (14) found that the use of 1 per cent K_2HPO_4 (0.57M) greatly increased lesion formation on plants of *Phaseolus vulgaris* L. inoculated with any of several viruses. In the present experiments with potato virus Y, mixtures of K_2HPO_4 and KH_2PO_4 at concentrations greater than 0.005M had an appreciable depressing effect on lesion formation. That K_2HPO_4 had no specific beneficial effect was shown by the fact that when the diluent was composed largely of this salt, as was the case when mixtures of pH 8 or above were used (Fig. 2), lesion formation was depressed. On the other hand, results with borate were similar to Yarwood's results with phosphate. In both cases there appeared to be a direct correlation between phytotoxicity and enhancement of lesion formation.

There are several disadvantages inherent in the use of *Physalis floridana* as a test plant for virus Y. About 3 months are required for the production of plants suitable for use. During this time the palnts will not withstand crowding, hence considerable bench space is required. The plants require considerable attention for they must be kept actively growing during the entire growing period and supplemental light is frequently necessary. If inoculated plants are exposed to temperatures much above 70° F., lesion formation will be poor or non-existent. Plants are susceptible to several other viruses and the presence of some of them in the inoculum may lead to erroneous conclusions. These disadvantages may limit the amount of work that can be done and may prevent use of the plant during part of the year or in certain regions, but they do not prohibit its use. With proper care, satisfactory results can be obtained. Until a better test plant is discovered,

Physalis floridana should make possible many studies on virus Y that previously have not been possible.

SUMMARY

Satisfactory lesion formation was obtained on plants of *Physalis floridana* inoculated with potato virus Y during early flowering, provided the plants had been kept growing vigorously and had been given supplemental light if grown during winter months. Lesion formation was increased by the use of carborundum, by shading, and by post-inoculation temperatures between 65° and 70° F. No lesions formed at 80° F. or above. A rapid progressive reduction in lesion count resulted from the continued use of inoculating-pads without renewal of the inoculum, from excessive rubbing, and often from increases in the length of time between inoculation and rinsing of leaves. Although borate buffers caused severe injury to leaves unless removed by rinsing within a minute following inoculation, the use of 0.1M borate (pH 8) resulted in more lesions than did that of any other diluent. This was followed in order by 0.1M glycylglycine (pH 7), 0.005 to 0.01M phosphate (pH 7) and water. Inocula differing in virus concentration by 20 or 10 per cent regularly produced statistically significant differences in lesion count when 36 or 72 half-leaves, respectively, were rubbed with each inoculum. When 2 inocula differing in virus concentration by a known amount were compared, each at 2 dilutions, the virus content of one relative to that of the other was regularly estimated with an error seldom exceeding 10 per cent.

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LITERATURE CITED

1. BAWDEN, F. C., AND F. M. ROBERTS. 1947. The influence of light intensity on the susceptibility of plants to certain viruses. Ann. Appl. Biol. **34**: 286-296.
2. BAWDEN, F. C., AND F. M. ROBERTS. 1948. Photosynthesis and predisposition of plants to infection with certain viruses. Ann. Appl. Biol. **35**: 418-428.
3. COCHRAN, W. G., AND GERTRUDE M. COX. 1950. Experimental designs. John Wiley and Sons, Inc. New York. 454 pp.
4. DARBY, J. F., R. H. LARSON AND J. C. WALKER. 1951. Variation in virulence and properties of potato virus Y strains. Wis. Agr. Exp. Eta. Res. Bul. 177. 32. pp.
5. HOLMES, F. O. 1942. Quantitative measurement of a strain of tobacco-etch virus. Phytopathology **32**: 1058-1067.
6. PRICE, W. C. 1945. Accuracy of the local-lesion method for measuring virus activity. IV. Southern bean mosaic virus. Amer. Jour. Bot. **32**: 613-619.
7. PRICE, W. C., AND E. L. SPENCER. 1943. Accuracy of the local lesion method for measuring virus activity. II. Tobacco-necrosis, alfalfa-mosaic, and tobacco-ringspot viruses. Amer. Jour. Bot. **30**: 340-346.
8. ROSS, A. F. 1948. Local lesions with potato virus Y. Phytopathology. **38**: 930-932.
9. ROSS, A. F. 1949. Factors affecting lesion formation on Physalis floridana inoculated with potato virus Y. (Abs.) Phytopathology **39**: 20-21.
10. ROSS, A. F. 1950. Local lesion formation and virus production following simultaneous inoculation with potato viruses X and Y. (Abs.) Phytopathology **40**: 24.
11. ROSS, A. F. 1950. Unrelatedness of potato virus Y and cucumber mosaic virus. Phytopathology **40**: 445-452.
12. SHERWOOD, M. B. 1947. Simple formulas for calculating percentage potency in three- and four-dose assay procedures. Science **106**: 152-153.
13. SPENCER, E. L., AND W. C. PRICE. 1943. Accuracy of the local-lesion method for measuring virus activity. I. Tobacco-mosaic virus. Amer. Jour. Bot. **30**: 280-290.
14. YARWOOD, C. E. 1952. The phosphate effect in plant virus inoculations. Phytopathology **42**: 137-143.
15. YOUDEN, W. J. 1937. Dilution curve of tobacco-mosaic virus. Contr. Boyce Thomp. Inst. **9**: 49-58.

OCCASIONAL TRANSMISSION OF SOME PLANT VIRUSES THROUGH THE EGGS OF THEIR INSECT VECTORS

L. M. Black [1]

Only 2 plant viruses, rice stunt virus (*Fractilinea oryzae*) Holmes (5) and clover club-leaf virus (*Aureogenus clavifolium* Black) have been reported to pass through the eggs of their insect vectors to the progeny. It is the purpose of this paper to report 2 additional cases in which this occurs. However, in the 2 instances to be described, transmission through the vectors' eggs is rare, a condition in marked contrast to that existing in rice stunt and clover club-leaf virus.

Both rice stunt and clover club-leaf viruses pass through a high percentage of the eggs of viruliferous leafhopper vectors to their progeny. The percentage of insect progeny transmitting virus to plants does not give an accurate measure of the proportion carrying either of these viruses. Fukushi (4) found some leafhoppers that produced viruliferous progeny, whereas they themselves failed to infect any plants although each had been transferred to a fresh susceptible rice seedling every day. Black (data to be published) found that some female leafhoppers tested individually for 3 weeks on each of 3 successive crimson clover plants, failed to infect any of them, yet produced progeny carrying clover club-leaf virus. Probably in these instances the best method of measuring the percentage of transovarian passage of virus is to consider only those females whose only source of virus was their mother and whose progeny were tested for virus without having an opportunity to acquire virus from plants. Of 18 such individuals recorded in Fukushi's chart on p. 143 (3) all or 100 per cent received virus through the egg. Of the 21 families selected without reference to virus and recorded in the main line of descent in Black's (2) study of clover club-leaf, only 1 instance occurred where the mother of the family failed to receive virus through the egg. In other words, the transovarian passage of virus was 95 per cent.

In all other cases tested, no transovarian passage has been reported. The experiments described below were designed to detect a small percentage of transovarian passage of wound-tumor virus, *Aureogenus magnivena* Black, and New Jersey potato yellow-dwarf virus, *Aureogenus vastans* H. var. *agalliae* Black. In detecting small percentages of transovarian passage, it is essential to eliminate the possibility of the progeny's acquiring small amounts of virus from plants on which they are reared. Adequate control of such a possible source of error may be obtained by using on the same plant at the same time vector groups that can be distinguished from each other by size, genetic marking, or some other means.

Teitelbaum and Goulet (6) described a maroon variant of *Agalliopsis novella* (Say) which is readily distinguished in nymph and adult stages from the normal insect. The maroon color was shown to be determined by a single recessive autosomal gene. In the following experiment this colored variant of the leafhopper was employed to distinguish control and test lots of insects. Fourteen maroon ovipositing females that had been reared from time of hatching on wound-tumor crimson clover plants (*Trifolium incarnatum* L.) were placed on each of the Grimm alfalfa (*Medicago sativa* L.) plants 1–3. Alfalfa is immune from this virus. The same number of ovipositing, non-viruliferous normal females were placed on each plant. Males were excluded. The plants were grown in 4-in. pots of soil that had been steamed to kill weed seeds. No weeds were present in any of the cages when the progeny were collected 55 days later. The maroon and normal progeny were separated and equal numbers from each alfalfa plant were tested in colonies of 10 on crimson clover plants for 2 weeks. Each colony was then transferred to a second test plant for 3 weeks. Test plants were observed in a greenhouse during 6 weeks. Of 25 maroon colonies, 3 were infective, 2 infecting 1 test plant and 1 both test plants. None of 25 normal colonies was infective.

At the same time, the experiment was duplicated on alfalfa plants 4-6 with viruliferous normal females and non-viruliferous maroon females. In this case, of 19 colonies of normal progeny, 7 were infective, 5 infecting 1 test plant and 2 both test plants. None of 17 colonies of maroon progeny from alfalfa plants 4-6 were infective. It is concluded that a small percentage of transovarian passage of wound-tumor virus occurs in *Agalliopsis novella*.

There is some indication that individual females may vary in the amount of transmission occurring through their eggs. Of 11 colonies from cage 5, 6 proved infective. Of 33 colonies from cages 1, 2, 3, 4 and 6, only 4 proved infective. The odds are greater than 50:1 that such a difference was not due to chance alone. Because the parent females had been treated as much alike as possible and the individuals in each category had been taken at random from a single collection for addition to alfalfa plants 1–6, it seems probable that a single female in cage 5 differed from the other leafhoppers, possibly genetically, in such a way as to occasion a higher percentage of transovarian passage.

In this experiment all but 1 transmitting colony infected the second test plant and as the average number of insects placed on the second test plant for all

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colonies was 7, as it was also for those from cage 5, the best estimate[3] of the percentage of transovarian passage is based on a colony number of 7 insects. In cage 5 the amount of transovarian passage was approximately 10.7 per cent; in the other cages, it was approximately 1.8 per cent.

A similar experiment was carried out to test for occasional transmission of New Jersey potato yellow-dwarf virus through the egg of the vector *Agallia constricta* Van Duzee. Twenty ovipositing females that had been reared from early nymphal stages on diseased crimson clover plants were placed on each of 6 Grimm alfalfa plants. Alfalfa is believed to be immune from the virus. Two weeks later the adults were removed and 80 non-viruliferous control nymphs of medium size (M) were added to each alfalfa plant. M nymphs were also added to a crimson clover plant in the acute and another in the chronic stage of the disease. Eleven days later all the M nymphs were collected from the 6 alfalfa plants as were the newly hatched nymphs (N). There were no weeds in the cages containing the alfalfa. From each alfalfa plant the same number of M and N colonies of 10 insects each, was tested on crimson clover. In some cases this was 6 colonies and in others 7; altogether 40 M colonies and 40 N colonies were tested. Each colony was tested for 2 weeks on each of 3 successive crimson clover plants. None of the M colonies from alfalfa infected any plants, although 20 control colonies of 5 M insects each, from the diseased crimson clover plants, infected 56 of 60 similar test plants. Of the 40 N colonies, only 3 infected test plants. One colony infected only 1 test plant, another infected 2 and the third infected all 3. Survival throughout the test was very good and the best estimate of the transovarian passage, based on 10 insects per colony, was about 0.8 per cent.

[3] The author is indebted to Dr. W. J. Youden for the method of calculation (1).

SUMMARY

Females of *Agalliopsis novella* reared on crimson clover plants infected with wound-tumor virus oviposited eggs which produced about 1.8 per cent of infective progeny. Evidence was obtained that occasionally a female occurs which has a much higher rate of transovarian passage of this virus. Females of *Agallia constricta* reared on crimson clover infected with New Jersey potato yellow-dwarf virus oviposited eggs which produced about 0.8 per cent of infective progeny.

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LITERATURE CITED

1. Black, L. M. 1941. Further evidence for multiplication of the aster-yellows virus in the aster leaf hopper. Phytopathology **31**: 120-135.
2. Black, L. M. 1950. A plant virus that multiplies in its insect vector. Nature **166**: 852-853.
3. Fukushi, T. 1939. Retention of virus by its insect vectors through several generations. Proc. Imp. Acad. (Japan) **15**: 142-145.
4. Fukushi, T. 1940. Further studies on the dwarf disease of rice plant. Jour. Faculty Agr., Hokkaido Imp. University **45**: 83-154.
5. Holmes, F. O. 1948. The filterable viruses. Bergey's Manual of Determinative Bacteriology Ed. 6. Williams & Wilkins, Baltimore. p. 1127-1286, I-XXIII.
6. Teitelbaum, Shirley S. and Pauline Goulet. 1950. A color mutant of the leafhopper Agalliopsis novella (Say). Proc. Ent. Soc. of Washington **52**: 269-271.

A STRAIN OF YELLOW BEAN MOSAIC VIRUS PRODUCING LOCAL LESIONS ON TOBACCO[1]

H. Rex Thomas and W. J. Zaumeyer

A reddish-brown blotching of bean pods, sometimes accompanied by mottling of leaves and necrosis of stems, has been frequently observed in varieties of pole snap bean, *Phaseolus vulgaris* L., grown in Idaho, Oregon and Washington. The red-node and yellow-dot viruses and what have been assumed to be strains of yellow bean mosaic virus have been isolated from plants with these symptoms. In preliminary greenhouse tests, one isolate obtained in 1950 from a strain of Blue Lake bean grown near Twin Falls, Idaho, appeared to belong in the yellow bean mosaic virus group, but it produced chlorotic local lesions on Turkish tobacco, a response atypical of viruses in this group.

The studies presented here deal with the identification of this isolate. The virus is classified as the severe yellow-mosaic strain of yellow bean mosaic virus.

MATERIALS AND METHODS.—All investigations were made in the greenhouse at Beltsville, Maryland, at temperatures of 21° to 27° C. The virus was maintained on plants of Pinto U. I. 72 bean[2]. Carborundum was used as an abrasive in all transfers. Plants were inoculated in the early stages of growth and in most cases the symptoms described are from plants grown 3–6 weeks in 4-in. pots in the host-range studies. When symptoms were not visible an attempt was made to recover the virus by inoculating either Pinto U. I. 72 or Stringless Green Refugee bean plants.

The viruses used in these studies were obtained from several sources: a culture of yellow bean mosaic virus believed to be representative of the type (Fig. 1, B) described originally by Pierce (7) was received from F. P. McWhorter, Oregon State College, Corvallis, Oregon; Ladino clover yellow-patch virus from K. W. Kreitlow; tobacco ringspot virus from H. H. McKinney; and a necrotic-lesion strain of yellow bean mosaic virus (8), a strain of cucumber mosaic virus systemically infectious to beans (1), common bean mosaic virus, and southern bean mosaic virus from a virus collection of the Bean Project, Plant Industry Station, Beltsville, Maryland.

SYMPTOMS AND HOST RANGE.—Bean: In the field the pods of infected Blue Lake plants had necrotic reddish-brown blotches on the surface and an internal darkening of the vascular strands. There were veinal necroses on some of the leaves and necroses of the stems. The plants were stunted and many leaves reduced in size, curled, and mottled.

In the greenhouse the pods on all the susceptible bean varieties were severely misshapen (Fig. 1, E), but no reddish-brown pod blotching characteristic of the field-infected plants was obtained. Symptoms on inoculated bean plants differed with the variety and, for convenience, are placed in 6 groups as follows.

On Blue Lake, Blue Lake 228, and Kentucky Wonder a few solid necrotic lesions developed in 5–6 days on the primary leaves, followed by veinal necrosis, by general chlorosis, and later by abscission. The necrosis gradually spread throughout the plant, finally killing it.

On Great Northern U. I. 15, Pinto U. I. 72, Idaho Pinto, and Michelite beans, reddish-brown lesions were followed by slight veinal necrosis and epinasty of the inoculated primary leaves, general stunting of the plant, curling of the trifoliate leaves, and pronounced yellow mottle and some yellow spotting of new leaves.

Infection on the varieties Stringless Black Valentine, Stringless Green Refugee, Topcrop, Sensation Refugee 1066 and Idaho Refugee was characterized by marked epinasty of the inoculated primary leaves (Fig. 1, I) followed by severe chlorosis and general breakdown (Fig. 1, D), curling of the growing point, development of an abscission layer at the pulvini of the leaflet and petiole, terminal killing, and death. Local lesions developed on Stringless Green Refugee, Sensation Refugee 1066 and Idaho Refugee.

On Bountiful, Florida Belle, Tendergreen, Full Measure and Contender varieties strong epinasty of the inoculated primary leaves was followed by chlorosis, but no veinal necrosis or abscission of the trifoliate leaves. The growing point was not killed but new growth was severely mottled (Fig. 1, A) and the plants were stunted.

On Unrivalled Wax, numerous, small, necrotic lesions were followed by slight veinal necrosis of the inoculated leaves and very mild systemic mottle.

No infection was obtained on Great Northern U. I. 16, 31, and 123.

Pea, *Pisum sativum* L. On Thomas Laxton and Perfected Wales, a yellow vein clearing appeared first, followed by yellow mottle, slight vein banding, and necrotic streaking of the stem.

Tobacco, *Nicotiana tabacum* L., *N. rustica* L., and *N. sylvestris* Speg: Numerous chlorotic local lesions, 1 to 3 mm. in diameter, developed 6–8 days after inoculation (Fig. 1, C). There was no systemic infection.

Other *Leguminosae*: Soybean, *Glycine max* (L.) Merrill var. Patoka and Blackhawk; broadbean, *Vicia faba* L. var. Broad Windsor—severe mottling.

Asparagus bean, *Vigna sesquipedalis* (L.) Fruwirth: very mild mottle.

Moth bean, *Phaseolus aconitifolius* Jacq. and guar, *Cyamopsis tetragonoloba* (L.) Taub: Numerous small necrotic lesions, no systemic infection.

Tepary bean, *P. acutifolius* A. Gray var. *latifolius* Freeman: systemic mottle, leaf cupping and terminal

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[2] The initials U. I. indicate that the strain was originated by the University of Idaho.

killing.

Jackbean, *Canavalia ensiformis* L. (DC.): Numerous necrotic lesions followed by general veinal necrosis of the inoculated leaves and slight malformation and veinal necrosis of trifoliate leaves.

White sweet clover, *Melilotus alba* Desr: Yellow mottled areas circular or elongated each with a center of normal green, later usually having a necrotic pin-

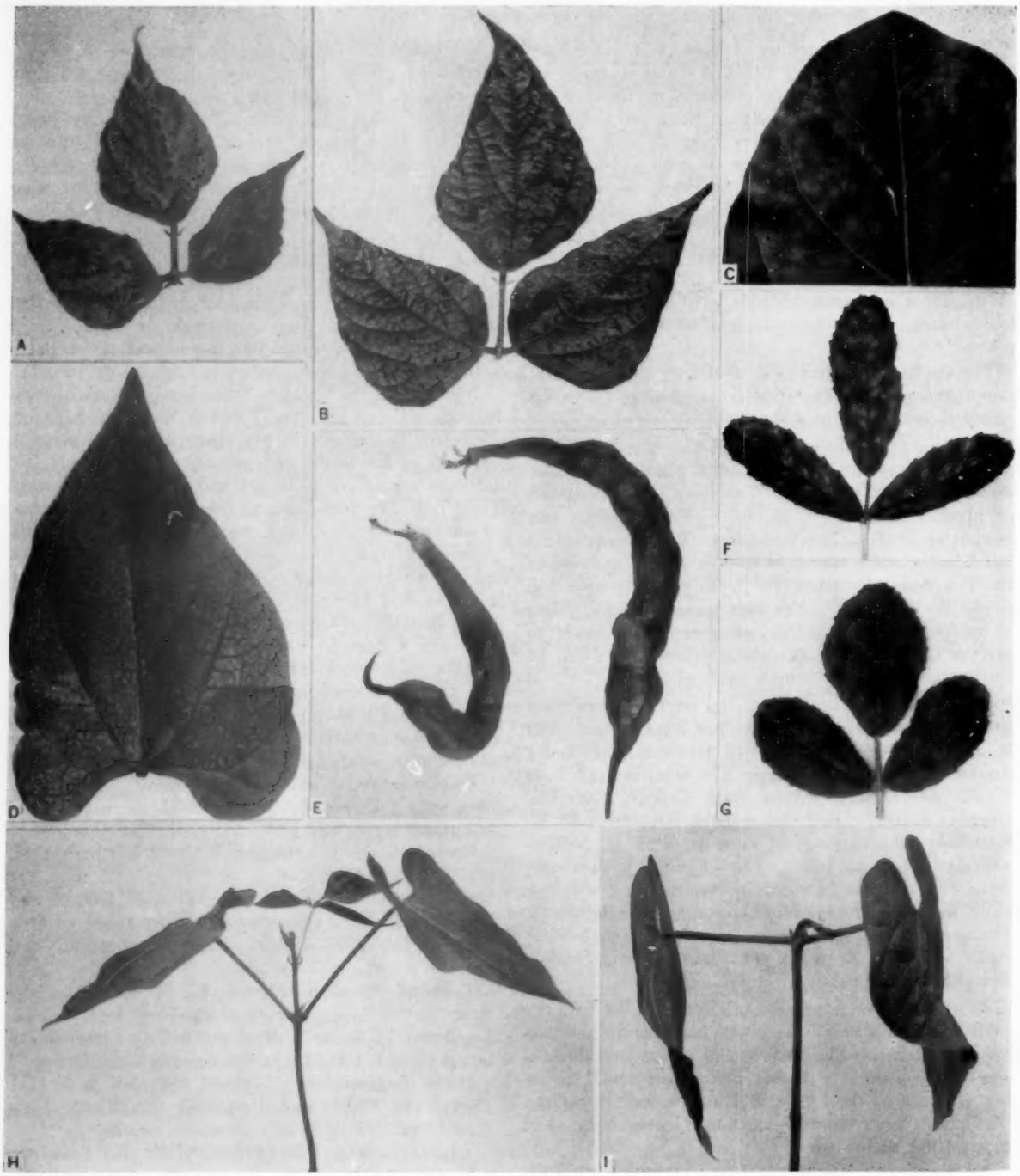

FIG. 1. A and B) comparison of severe yellow mosaic and type strains of yellow bean mosaic on Bountiful bean leaves of the same age. A) Severe mosaic, causing extreme mottling and reduction in leaf size. B) Type strain, causing moderate leaf mottle. C-F) Severe-mosaic virus symptoms produced on certain hosts. C) Chlorotic local lesions on tobacco leaf. D) Inoculated primary leaf of Stringless Green Refugee bean. E) Misshapen pods from infected plant of Topcrop bean. F, G) Systemic infection on white sweet clover leaves. H) Healthy Stringless Green Refugee bean plant. I) Epinasty of inoculated primary leaves and tip curvature on Stringless Green Refugee bean.

point center with a small chlorotic halo (Fig. 1, F, G). The necrotic area may enlarge to 1 mm. in diameter on older leaves.

Crimson clover, *Trifolium incarnatum* L: Intense yellow mottle and severe stunt.

Ladino clover, *T. repens* L. f. *giganteum* Lagr.-Foss: mild mottle.

No infection was observed on the following: Spinach, *Spinacia oleracea* L. var. Bloomsdale; cucumber, *Cucumis sativus* L. var. Cubit; zinnia, *Zinnia elegans* Jacq. var. California Giant; cabbage, *Brassica oleracea* var. *capitata* L. var. Jersey Wakefield; snapdragon, *Antirrhinum majus* L. var. Tall Giant; tomato, *Lycopersicon esculentum* Mill. var. Marglobe; jimson weed, *Datura stramonium* L.; *Nicotiana glutinosa* L.: lima bean, *Phaseolus lunatus* L. var. Triumph; runner bean, *P. coccineus* L. var. Streamliner; red clover, *Trifolium pratense* L.; alfalfa, *Medicago sativa* L.; pea, *Pisum sativum* L. var. Rogers Perfection, Wisconsin Perfection, and Associated Seed Growers Resistant Early Perfection 326.

PROPERTIES.—Expressed juice from young Stringless Green Refugee plants was used for all studies of physical properties and results were based upon systemic infection in this variety. Twenty plants were inoculated for each test.

The virus was inactivated in 10 minutes at 50°-55°C. It withstood dilution of 2000× but not 3000× and was infectious after storage for 48 hours *in vitro* at 18° C., but not after 72 hours.

CROSS PROTECTION.—Fifteen to 20 plants of Stringless Green Refugee were inoculated with each of the following viruses: common bean mosaic, necrotic-lesion strain of yellow bean mosaic, and a strain of cucumber mosaic systemically infectious on beans. After the systemic symptoms of the several viruses had developed on the inoculated plants, 10 plants infected with each virus were inoculated with severe-yellow-mosaic virus. Approximately 14 days later terminal necrosis, characteristic of severe yellow mosaic, developed on all plants except those infected with common bean mosaic. However, 10 days later these plants also developed terminal necrosis. The only check plants that showed any necrosis were those inoculated with severe-yellow-mosaic virus.

Trifoliate leaves of Pinto U. I. 72 systemically infected with severe-yellow-mosaic virus were inoculated separately with the necrotic-lesion strain of yellow bean mosaic, tobacco ring-spot, southern bean mosaic, and Ladino clover yellow-patch viruses. Thirteen days later local lesions typical of the 4 viruses developed on the leaves infected with severe yellow mosaic virus as well as on the leaves of the previously uninoculated check plants.

Healthy Unrivalled Wax bean plants and some that has been systemically infected with the necrotic-lesion strain of yellow bean mosaic virus were inoculated with the severe-yellow-mosaic virus. Ten days later, primary lesions typical of severe mosaic developed on the inoculated leaves of both groups of plants.

Mottled leaves of broadbean infected with severe-yellow-mosaic virus and leaves of healthy plants were inoculated with the necrotic-lesion strain. Seven days later local lesions developed on the leaves of both groups of plants.

These results suggest that on the basis of cross protection severe-yellow-mosaic is not related to the following viruses: southern bean mosaic, a strain of cucumber mosaic systemically infectious to bean, tobacco ring-spot, Ladino clover yellow-patch, common bean mosaic, and the necrotic-lesion strain of yellow bean mosaic.

DISCUSSION.—Host-range and property studies suggest that the severe-yellow-mosaic virus is in the yellow bean mosaic group. Lack of cross protection by severe-mosaic virus, common bean mosaic virus, and the necrotic-lesion strain of yellow bean mosaic virus is evidence to the contrary. However, this is not considered sufficient to exclude severe-yellow-mosaic virus from the yellow bean mosaic virus group, mainly because of the similarity of symptoms produced on many bean varieties by severe-yellow-mosaic virus and certain strains of yellow bean mosaic virus. The failure of severe-yellow-mosaic virus to infect Great Northern U. I. 123 bean, Wisconsin Perfection pea and runner bean is a characteristic in common with yellow bean mosaic virus.

The severe-yellow-mosaic virus can readily be distinguished from previously described strains of yellow bean mosaic virus. In Table 1 are listed symptoms on certain hosts, helpful in distinguishing several of the previously reported strains of the yellow bean mosaic virus (2, 3, 4, 5, 6, 7, 8, 9, 10). The necrotic-lesion and severe-yellow-mosaic strains are the only 2 reported infectious on tobacco. The former virus produces large, white local rings on the inoculated leaves while severe-yellow-mosaic virus produces numerous chlorotic local lesions 1–3 mm. in diameter. Other rapid means of distinguishing these 2 viruses are as follows: (1) the necrotic-lesion strain produces solid brownish-red necrotic local lesions on broadbean while the severe-yellow-mosaic virus produces systemic mottle; (2) the former virus produces local lesions on Great Northern U. I. 123, while severe-yellow-mosaic virus is not infectious to this variety and (3) the necrotic-lesion strain causes no pod malformation while severe-yellow-mosaic virus produces it.

Pod-distorting and severe-yellow-mosaic viruses are similar in that both cause severely misshapen pods and systemic necrosis on many bean varieties. Pod-distorting virus does not infect broadbean, soybean, or tobacco whereas severe-yellow-mosaic virus infects these hosts.

Isolate 1, a strain of yellow mosaic virus, reported by Hagedorn (3) can be distinguished from severe-yellow-mosaic virus by not infecting tobacco. The type strain of yellow mosaic virus differs from severe-yellow-mosaic virus in producing much milder symptoms on most hosts. Severe-yellow-mosaic virus produces considerable necrosis on many bean varieties

TABLE 1.—*Comparison of symptoms produced by strains of the yellow bean mosaic virus on various species*[a]

Species or variety	Type[b]	Pod-distorting	X	Isolate 1	Necrotic lesion	Severe yellow mosaic
Beans						
Blue Lake	Mottle, slight stunt, red nodes	Top necrosis	Necrosis	Necrosis	Mottle, some necrosis	Necrosis
Bountiful	Mottle, stunt	Top necrosis	——	——	Mottle	Mottle, stunt
Great Northern U.I. 123	Faint mottle	None	——	——	Local lesions	None
Michelite	Mottle, stunt	Top necrosis	——	Stem necrosis, mottle no top necrosis	Local lesions, mottle	Severe mottle, some top necrosis
Peas						
Wisconsin Perfection	None	None	None	None	None	None
Perfected Wales	Distinct yellow mottle	Yellow mottle, vein clearing	[c]	Mottle	None	Mottle
Crimson clover	Vein clearing, reduction in leaf size	Yellowish green mottle vein clearing	Mottle	Mottle, dwarfing	None	Mottle, dwarfing
Red clover	None	None	Mottle	Mottle	None	None
Broadbean	Light greenish yellow mottle	None	Mottle	Mottle	Local lesions	Mottle
Cowpea	None[d]	——	——	None	Local lesions, mottle	None
Asparagus bean	None[d]	——	——	——	Local lesions, mottle	Mottle
Soybean	Mottle	None	——	Chlorosis	Mottle	Mottle
Tobacco	None	None	——	None	White local rings	Chlorotic locals

[a] Except those for necrotic-lesion and severe-mosaic strains the data are based on previously published reports.
[b] Taken from Grogan and Walker (2).
[c] X virus reported on various pea varieties (5).
[d] Taken from Zaumeyer and Wade (10).

while the type strain does not. An adequate comparison with the X strain (5) is not possible because of the limited published data.

The thermal inactivation point of the severe-yellow-mosaic strain, 50° to 55° C., is the lowest reported for any of the strains of yellow bean mosaic virus although McWhorter (6) reported strains with thermal inactivation points of 56°, 57° and 58° C. The type strain was inactivated between 58° and 60° C.

If yellow bean mosaic virus should become a consistent serious factor in bean production in any area, it should be noted that Great Northern U. I. 123 has been reported resistant to systemic infection by 3 of the strains. The new Great Northern varieties U. I. 16 and U. I. 31 are also resistant to the severe-yellow-mosaic and necrotic-lesion strains and probably to the pod-distorting strain. These 2 varieties might be good sources of resistance as they are also resistant to curly top, common bean mosaic, and the strain of common bean mosaic infectious to Great Northern U. I. 15, Robust, and Michelite beans.

SUMMARY

Severe-yellow-mosaic virus, which causes strong epinasty, chlorosis, and veinal necrosis of the inoculated primary leaves followed by top necrosis and death of certain bean varieties and severe systemic mottle in others was isolated from a bean plant in Idaho.

The virus was infectious to all varieties of beans tested with the exception of Great Northern U. I. 123, 31, and 16.

Except for the yellow necrotic-lesions on *Nicotiana tabacum*, *N. rustica* and *N. sylvestris*, no infection was obtained outside the Leguminosae.

The virus was inactivated between 50° and 55° C. in 10 minutes and at 18° C. between 48 and 72 hours. It withstood dilution of 2000× but not 3000×.

No cross protection against severe-yellow-mosaic virus was obtained from viruses of common bean mosaic, the necrotic-lesion strain of yellow bean mosaic, Ladino clover yellow-patch, tobacco ring-spot, southern bean mosaic, and a strain of cucumber mosaic.

Because of the similarity of properties and particularly of host range and of symptoms produced on beans the virus is classified as severe-yellow-mosaic strain of yellow bean mosaic virus.

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LITERATURE CITED

1. DOOLITTLE, S. P. AND W. J. ZAUMEYER. 1952. A ring-spot disease of peppers caused by strains of cucumber mosaic virus from pepper and alfalfa. (Abs.) Phytopathology **42**: 7.
2. GROGAN, R. G. AND J. C. WALKER. 1948. A pod-distorting strain of yellow mosaic virus of bean. Jour. Agr. Res. (U.S.) **77**: 301-314.
3. HAGEDORN, D. J. 1950. The relation of bean virus 2 to pea mosaic in Wisconsin. Phytopathology **40**: 684-698.
4. HOUSTON, B. R., AND J. W. OSWALD. 1951. Mosaic diseases of Ladino clover in California (Abs.) Phytopathology **41**: 940.

5. McWhorter, F. P. and L. Boyle. 1946. X-disease and other forms of yellow bean mosaic in Western Oregon. Oregon Agr. Exp. Sta. Cir. of Infor. No. 379.
6. McWhorter, F. P. 1949. Susceptibility of selections of Perfection peas to strains of yellow bean mosaic. U. S. Dept. Agr. Pl. Dis. Rptr. **33**: 139-144.
7. Pierce, W. H. 1934. Viroses of the bean. Phytopathology **24**: 87-115.
8. Zaumeyer, W. J. 1952. A new necrotic-lesion-producing strain of yellow bean mosaic. (Abs.) Phytopathology **42**: 24.
9. Zaumeyer, W. J. A new necrotic-lesion-producing strain of yellow bean mosaic. Phytopathology **43**: 38-42.
10. Zaumeyer, W. J. and B. L. Wade. 1935. The relationship of certain legume mosaics to bean. Jour. Agr. Res. (U.S.) **51**: 715-749.

STUDIES ON BAYBERRY YELLOWS [1]

S. P. Raychaudhuri [2]

A yellows-type disease on bayberry plants (*Myrica carolinensis* Mill.) in the blueberry (*Vaccinium corymbosum* L.) and cranberry (*Vaccinium macrocarpon* Ait.) growing areas near Pemberton, New Jersey, was observed in 1950 by Dr. L. O. Kunkel who transmitted the virus to carrot (*Daucus carota* L.) and periwinkle (*Vinca rosea* L.) plants by dodder (*Cuscuta campestris* Yuncker). Symptoms in these plants indicated that the disease which he designated bayberry yellows was not caused by the viruses of blueberry stunt nor false blossom of cranberry.[3] Raychaudhuri (15) reported in a preliminary account that in bayberry-yellows-affected periwinkles symptoms were not identical with those of any of the known yellows diseases studied earlier in this host.

The writer observed bayberry disease in 2 locations near Pemberton, N. J. but could not find it in the vicinity of Yonkers, New York or near Bear Mountain in New York State. The disease was characterized by yellowing, stunting, and often by a witch's-broom appearance (Fig. 1). Affected plants were normally sterile but in some instances bore a few healthy shoots with normal fruits. Severely affected plants could be recognized from a considerable distance since they were very much stunted and their leaves were reduced in size.

Materials and methods.—All periwinkle, cranberry, and blueberry plants under experiment were grown from cuttings belonging to the same stock of healthy clones, respectively. Healthy dodder of the species *Cuscuta campestris* Yuncker and *C. subinclusa* Dur. and Hilg. was obtained from Dr. L. O. Kunkel who also kindly supplied a periwinkle plant affected by the bayberry yellows virus. Potato plants were raised from virus-free tubers of the Katahdin variety obtained from Dr. E. S. Schultz of the Bureau of Plant Industry, U. S. Department of Agriculture, Beltsville, Maryland. Healthy bayberry cuttings were used for transmission experiments. Later, bayberry seedlings were grown from true seeds that had been collected from healthy plants. Plants of other species were raised from seeds purchased from commercial seed houses unless otherwise stated. Insects collected from the Pemberton area and *Macrosteles divisus* Uhler obtained from a virus-free colony maintained at the Rockefeller Institute for Medical Research were used in transmission experiments. In all insect tests plants were covered with celluloid cages of suitable size. In some instances insects were fed on single leaves by means of the insect-vector cage described by Maramorosch (14).

Transmission of the virus.—*Transmission by grafting.*—Cleft-grafts were used in all grafting experiments. The virus was easily transmitted in the greenhouse by grafting from diseased periwinkles to 46 healthy periwinkle plants. Symptoms appeared in these plants within 32-47 days. Diseased scions obtained from infected bayberry plants at Pemberton were grafted to healthy stocks of 4 bayberry plants in a greenhouse. Three of these showed disease symptoms after 56 to 96 days. Scions of diseased petunia plants (*Petunia hybrida* Vilm. var. *nana compacta* (Cream Star)) that had been infected by means of dodder removed from diseased periwinkle plants were grafted to healthy stocks of *Petunia hybrida, Lycopersicon esculentum* Mill. (tomato), *Solanum tuberosum* L. (potato), *Nicotiana tabacum* L., *N. glutinosa* L., and *N. rustica* L. All 12 petunia plants showed disease symptoms after 35–58 days but the others remained healthy.

Transmission by dodder.—Healthy plants of dodder, maintained by transferring branches routinely on healthy *Nicotiana glauca* Grah. plants, were transferred to bayberry and periwinkle plants infected with the bayberry-yellows virus. The dodder was trailed round the apical shoots of test plants and within 6–10 days had parasitized them. On periwinkle it flourished and grew well. On bayberry, however, it withered within 52–67 days. Dodder that had been growing on diseased bayberry or periwinkle plants for 3–4 weeks was transferred from each source to 5 healthy bayberry, 6 healthy periwinkle, and 24 healthy young carrot plants. The dodder was allowed to parasitize the test plants for a period of 45 days, after which it was removed. In the control experiment 3 healthy bayberry, 6 healthy periwinkle, and 12 healthy young

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[3] Personal communication.

carrot plants were used. *Cuscuta campestris* readily transmitted the virus from diseased periwinkle to healthy periwinkle and carrot plants. All periwinkle plants and 21 of 24 carrot plants became infected after 32–77 and 48–103 days, respectively. When attempts were made to transfer the virus from bayberry plants by dodder, only 1 of 5 bayberry plants, 3 of 6 periwinkles, and 7 of 24 carrot plants became diseased. Control plants observed for 28 weeks showed no disease symptoms.

A number of dodder-transmission experiments with plants of other species belonging to 11 families were carried out. One of 24 *Myrica carolinensis* Mill., 58 of 58 *Vinca rosea* L., 127 of 136 *Daucus carota*, 16 of 16 *Beta vulgaris* L. (sugar beet), 24 of 24 *Petunia hybrida* Vilm. var. *nana compacta* (Cream Star), and 19 of 24 *Allium cepa* L. (onion) plants became infected. The following species were not infected: *Callistephus chinensis* Nees (China aster), *Calendula officinalis* L., *Zinnia multiflora* L., *Medicago sativa* L. (alfalfa), *Phaseolus vulgaris* L., *Vicia faba* L., *Trifolium incarnatum* L. var. Bokhara (Tall Sweet), *Vaccinium corymbosum* L., *V. macrocarpon* Ait., *Gomphrena globosa* L., *Datura stramonium* L., *Solanum melongena* L., *S. tuberosum* L., *Lycopersicon esculentum* Mill., *Nicotiana tabacum* L., *N. glauca* Grah., *N. glutinosa* L., *N. rustica* L., and *Prunus persica* (L.) Stokes. Each test plant was under observation for 16 weeks.

In a limited number of tests transmission was also obtained by means of *Cuscuta subinclusa*.

Attempts to transmit the virus by mechanical means.—Extracts from severely affected young leaves of bayberry, periwinkle, and carrot plants were used to inoculate young leaves of 4 healthy bayberry, 6 periwinkle, 12 carrot, and 12 beet plants, employing 320-mesh carborundum as an abrasive and the usual rubbing method. The inoculated plants in each experiment were under observation for 20 weeks. None of them developed disease symptoms. The experiment was repeated with similar results.

Attempts to transmit the virus by insects.—The following insects, belonging to the Cicadellidae, were collected from affected bayebrry plants during the period June to October, 1951:[4] *Empoasca fabae* Harris, *Erythroneura* sp. (obliqua group), *Graphocephala coccinea* (Forster), *Gyponana octolineata* (Say), *Scaphytopius acutus* (Say), *Scaphytopius verecundus* (Van D.), *Scaphytopius* sp., *Scleroracus vaccinii* (Van D.), and *Scleroracus* sp., possibly new.

[4] The author is thankful to Dr. Edgar G. Rex and Dr. Martin T. Hutchinson for their cooperation in insect collection from Pemberton, New Jersey, and to Dr. C. F. W. Muesebeck of the U. S. Department of Agriculture, Washington, D. C., for identifying the leafhoppers.

Transmission experiments were attempted with those insects which survived a preliminary 2-week test feeding on blueberry, cranberry, alfalfa, China aster, periwinkle, or carrot plants. This preliminary test was undertaken to determine whether or not the leafhoppers were already carrying viruses to which the test plants were susceptible. None of the plants of the 6 species showed disease symptoms during a period of 104 days. Later, these insects were allowed to feed on bayberry-yellows-affected carrot and periwinkle plants for varying periods and were subsequently caged on healthy test plants. None of them transmitted bayberry yellows during the period of observation which ended in May, 1952.

In another experiment, *Macrosteles divisus* leafhoppers obtained from virus-free colonies were caged on carrot plants affected by bayberry yellows and after a 4-week feeding period were transferred to healthy aster and carrot plants. As a control, virus-free *M. divisus* were confined on carrot plants infected with aster yellows and then were transferred to healthy aster and carrot plants. *M. divisus* transmitted the aster-yellows virus from carrot to aster and carrot plants but failed to transmit the virus causing bayberry yellows.

Attempts to transmit bayberry yellows through seed.—*Bayberry seeds.*—Since severely diseased bayberry plants were sterile, seeds were collected from partially diseased plants in which only 1 or a few branches showed symptoms. They germinated readily when subjected to a period of after-ripening (1) at 5° C. or were placed in open, mulched coldframes during winter months (December to February) for a period of 10 weeks prior to placing the flats in the greenhouse. Of 50 seeds, 38 germinated (76 per cent) and all plants remained healthy for a period of 18 weeks when the experiment was ended.

Periwinkle seeds.—In severely diseased periwinkle plants the flowers were virescent and seeds did not develop. Hence, seeds were collected from branches borne by 3 partially diseased plants. Of 50 seeds, 42 germinated (84 per cent) within 3–5 weeks in the greenhouse in flats containing sand. All remained healthy for 18 weeks after which no further observations were made.

Dodder seeds.—Seeds were collected from *Cuscuta campestris* plants that had been growing for 8 months on a periwinkle plant affected by bayberry yellows. Of 100 seeds that were sown in the greenhouse in a flat containing sand, 52 germinated. Each of 26 of these dodder seedlings was allowed to parasitize a healthy young periwinkle plant and each of the other 26, a healthy young carrot plant. After 45 days all dodder was removed and destroyed. None of the test plants showed any disease symptoms up to the twen-

FIGS. 1–3, 5–7.—Fig. 1. a) Healthy shoot of bayberry. b) Bayberry shoot affected by yellows disease.—Fig. 2. Periwinkle flowers: the flower at the extreme right is healthy; other 5 flowers are diseased and show various degrees of malformation and virescence.—Fig. 3. A diseased periwinkle leaf showing thickened and wavy midrib.—Fig. 5. A carrot plant affected by bayberry-yellows virus.—Fig. 6. A petunia plant affected by bayberry-yellows virus.—Fig. 7. a) Bayberry-yellows-affected periwinkle cured by heat treatment. b) Bayberry-yellows affected periwinkle without heat treatment (control).

a
b
1
5
3
a
b
7
6
2

tieth week.

SYMPTOMS PRODUCED BY BAYBERRY-YELLOWS VIRUS ON DIFFERENT HOSTS.—*Myrica carolinensis.*—The first symptom visible on young apical leaves was a slight waviness of margins and tips. Leaves showed distortion of margins and apices, were pale, yellowish, much reduced in size, crowded and leathery. The internodes were shortened and the whole plant was stunted. Plants infected at an early age were sterile. However, a few fruits were formed on late-infected plants.

Vinca rosea.—The virus did not cause severe stunting of periwinkle plants. The first symptoms were clearing of veins, various types of malformation of leaves and flowers, and virescence of flowers (Fig. 2). The leaves later became yellowish and much reduced in size. Frequently in severely diseased leaves the midrib was very much thickened and wavy (Fig. 3). Petals of newly-formed blossoms were pale, white, and finally green. Frequently the branches were very thin and bore minute leaves. The style of diseased flowers was very short, green, and somewhat condensed. Frequently a new bud was borne by the pistil at its stigmatic end (Fig. 4) and from this a new virescent flower developed.

Daucus carota.—Leaves of young plants were chlorotic, much reduced in size and the petioles were somewhat twisted (Fig. 5). Occasionally the main crown was much condensed and a bushy type of growth developed. Roots were distorted, twisted, and much reduced in size.

Beta vulgaris.—Leaves were characteristically reduced in size, chlorotic, narrow, leathery and variously malformed. Sometimes only a thin wiry midrib was left with very narrow strips of lamina. The plant was considerably stunted.

Petunia hybrida.—Leaves were chlorotic, reduced in size, and plants were dwarfed (Fig. 6). Vein-clearing was observed in young leaves. Flowers were variously malformed and virescent.

Apium graveolens.—Plants were not reduced in size. Chlorosis on young leaves was the first symptom. Later the light yellow areas turned a deep yellow. Petioles were not twisted as in aster-yellows infection.

Tagetes signata.—Except that plants were stunted and chlorotic, the general habit of growth was not noticeably different from that of healthy plants. Flowers on severely infected plants were pale green and never opened.

Allium cepa.—Plants were severely dwarfed and leaves were chlorotic. Plants infected withered early and died in 76–112 days.

Cuscuta campestris.—Infected dodder, grown on aster plants immune from the virus, was reduced in diameter by about ⅓–½. Flowers and viable seeds were produced by diseased dodder.

Cuscuta subinclusa.—Due to infection with bayberry-yellows virus, this dodder, maintained on aster plants, was reduced in diameter by about ½. Normal flowers were produced.

RETENTION OF BAYBERRY-YELLOWS VIRUS IN CUSCUTA CAMPESTRIS.—To determine how long the bayberry yellows virus was retained by dodder, a piece of an infective branch (about 2 in. long) growing on a bayberry-yellows-affected periwinkle plant was transferred to an immune host, a young aster plant, in April, 1951. This stock was maintained by routinely transferring to young healthy aster plants. At weekly or biweekly intervals small branches of dodder were removed from each set of aster plants and transferred to 2 or 3 young healthy carrot plants. Each set of aster plants was under observation for 18 weeks. Dodder transfers were continued for 14 months. All the carrot plants, excepting 6 in 3 tests made in December, 1951 and January, 1952, showed disease symptoms. The dodder showed disease symptoms during this long period of 14 months. Hence, the virus was presumably retained in dodder over a period of 14 months.

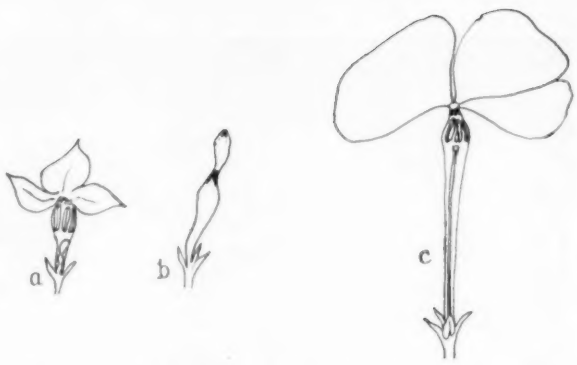

FIG. 4. a) Longitudinal section of a virescent periwinkle flower showing dwarfing. × 2; b) condensed style of a virescent periwinkle flower bearing a flower bud at its stigmatic end. × 4; c) Longitudinal section of a healthy periwinkle flower. × 2.

CURE OF THE DISEASE.—*Heat-treatment.*—The technique employed was the same as that described by Kunkel (9). Since bayberry plants failed to tolerate a temperature of 42° C., periwinkle plants with well developed symptoms of bayberry yellows were placed in a hot room on a table very close to a window. These test plants were 5–6 months old and about 1½ to 2 ft. high. They were held for from 1–14 days at 36.5°–37° C. and 41.5°–42° C., respectively. Two untreated plants in each test served as controls. After treatment the plants were removed to a greenhouse and observed for 9 months. Treated plants which produced healthy foliage and normal pink flowers (Fig. 7) over a period of 6 months or more were considered cured. Healthy periwinkle plants were grafted with scions from heat-treated plants in order to confirm whether or not the latter were actually cured. All tested plants kept 6 days or longer at 41.5°–42° C. were cured, while plants treated for shorter periods or at lower temperature remained diseased.

To determine whether or not cured periwinkle plants were immune from bayberry yellows, 2 plants cured by exposure to 41.5°–42° C. for 6 and 8 days, respectively, were grafted with diseased scions from periwinkles.

Within a period of 34–41 days, both showed symptoms of bayberry yellows. Hence, the cured periwinkles were not immune from bayberry yellows.

Ultraviolet radiation.—Diseased *Cuscuta campestris* and *C. subinclusa*, that had been growing on bayberry-yellows-affected periwinkles for 3–6 months, were irradiated by a Hanovi ultraviolet lamp having a spectrum at 2537 Å and running on 110 volts A.C. at a distance of 6 to 7 cm.[5] In 4 experiments with *C. campestris* and 2 with *C. subinclusa*, batches of 6 branches of dodder, approximately 2 in. long, were irradiated for 10 minutes, 1 hour, 2 hours, and 4 hours. Similar sets of healthy dodder were irradiated as controls. The treated dodder was then transferred to healthy young carrot plants and allowed to parasitize them for a period of 8 weeks after which it was removed and destroyed. Healthy or diseased dodder irradiated for 4 hours dried and withered on the carrot plants. The test plants bearing dodder that had been irradiated for 10 minutes, 1 hour, and 2 hours came down with bayberry-yellows symptoms in due course. This indicated that bayberry-yellows virus in dodder was not inactivated by exposure to ultraviolet light for periods up to 2 hours. All control plants remained healthy.

Discussion.—The virus causing the yellows disease of bayberry produced typical symptoms of the yellows type in all susceptible species which were tested. It also resembled the viruses causing yellows diseases in its inability to be transmitted mechanically or through seeds and also in its cure by heat treatments. Two species of dodder, *Cuscuta campestris* and *C. subinclusa*, transmitted it to susceptible hosts. In its host range, however, it showed distinctive characteristics. Among all the solanaceous species tried the virus infected only *Petunia hybrida*. No leguminous plant was infected.

Although the vector of the virus has not been determined thus far, negative results with *Macrosteles divisus*, *Scleroracus vaccinii* and *Scaphytopius verecundus* indicated that the virus of bayberry yellows differed from those of aster yellows (7), cranberry false blossom (4), and blueberry stunt (18). Unlike the California strain of aster-yellows virus, bayberry-yellows virus could not be transmitted to *Zinnia* (8). Symptoms produced on celery differed from those of the California strain of aster yellows (16). The inability of bayberry-yellows virus to infect potato plants indicated that it differed from potato witch's broom virus (11) and from alfalfa witch's broom (13). Also, bayberry yellows seemed to differ from big bud of tomato because during 7–8 weeks tomato plants that were grafted with diseased scions of *Petunia* did not become infected.

Bayberry-yellows virus was presumably retained in dodder growing on healthy plants over a period of 14 months. In this respect it behaved like the viruses causing cucumber mosaic (2) and cranberry false blossom (3, 12) but not like those causing tobacco mosaic or tobacco ringspot (6).

Bayberry-yellows disease was cured in periwinkles after exposure at 41.5°–42° C. for 6 days or more. At 36.5°–37° C. the disease could not be cured when plants were exposed for periods up to 14 days. Bayberry-yellows virus resembles aster yellows (10), potato witch's broom (11), cranberry false blossom (12), and alfalfa witch's-broom (13) viruses in its thermal inactivation point, although it differs from them in many other respects.

The virus causing the yellows disease of bayberry seems to differ from any previously described. It resembles closely members of the genus Chlorogenus (5) and is proposed as a new species and designated as *Chlorogenus myricae* sp. nov. The virus could also be designated as *Myrica* virus 1 according to Smith (17).

SUMMARY

A yellows-type disease was observed in bayberry (*Myrica carolinensis*) plants growing in Pemberton, New Jersey.

The virus was transmitted by cleft-grafting and also by the agency of dodder, *Cuscuta campestris* and *C. subinclusa*, but not by juice inoculation. Ten species of leafhoppers were tested but failed to transmit the virus, nor could it be transmitted through seeds of bayberry, periwinkle, or dodder.

The virus was transmitted to *Myrica carolinensis*, *Vinca rosea*, *Daucus carota*, *Beta vulgaris*, *Petunia hybrida* var. *nana compacta* (Cream Star), *Apium graveolens* var. Burpee's Fordhook, *Tagetes signata* var. *pumila* (Golden Yellow), *Allium cepa*, *Cuscuta campestris*, and *C. subinclusa*. The following species proved nonsusceptible: *Callistephus chinensis* (China aster), *Calendula officinalis*, *Zinnia multiflora*, *Medicago sativa* (alfalfa), *Phaseolus vulgaris*, *Vicia faba*, *Trifolium incarnatum* var. Bokhara (Tall Sweet), *Vaccinium corymbosum*, *V. macrocarpon*, *Gomphrena globosa*, *Datura stramonium*, *Solanum melongena*, *S. tuberosum*, *Lycopersicon esculentum*, *Nicotiana tabacum*, *N. glauca*, *N. glutinosa*, *N. rustica*, and *Prunus persica*.

In all species except dodder the virus caused typical yellows symptoms.

Bayberry-yellows virus was apparently retained by dodder over a period of 14 months, growing on healthy aster plants which are immune from the virus.

Bayberry yellows in periwinkle was cured by heat treatment at 41.5°–42° C. for 6 days or more, but not at 36.5°–37° C. when exposed up to 14 days.

Bayberry-yellows virus was not identical with any of the known yellows viruses.

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[5] Dr. George I. Lavin's cooperation in these experiments was much appreciated.

LITERATURE CITED

1. Barton, Lela V. 1932. Germination of bayberry seeds. Contrib. Boyce Thompson Inst. **4**: 19-25.
2. Bennett, C. W. 1940. Acquisition and transmission of viruses by dodder (Cuscuta subinclusa). (Abs.) Phytopathology **30**: 2.
3. Costa, A. S. 1944. Multiplication of viruses in the dodder, Cuscuta campestris. Phytopathology **34**: 151-162.
4. Dobroscky, Irene D. 1931. Studies on cranberry false blossom disease and its insect vector. Contrib. Boyce Thompson Inst. **3**: 59-83.
5. Holmes F. O. 1948. The filterable viruses. Bergey's Manual of Determinative Bacteriology. Ed. 6. Suppl. No. 2. The Williams and Wilkins Co., Baltimore, Md. pp. 1127-1286.
6. Johnson, F. 1941. Transmission of plant viruses by dodder. Phytopathology **31**: 649-656.
7. Kunkel, L. O. 1926. Studies on aster yellows. Amer. Jour. Bot. **13**: 646-705.
8. Kunkel, L. O. 1932. Celery yellows of California not identical with the aster yellows of New York. Contrib. Boyce Thompson Inst. **4**: 405-414.
9. Kunkel, L. O. 1936. Heat treatments for the cure of yellows and other virus diseases of peach. Phytopathology **26**: 809-830.
10. Kunkel, L. O. 1941. Heat cure of aster yellows in periwinkles. Amer. Jour. Bot. **28**: 761-769.
11. Kunkel, L. O. 1943. Potato witches'-broom transmission by dodder and cure by heat. Proc. Amer. Philos. Soc. **86**: 470-475.
12. Kunkel, L. O. 1945. Studies on cranberry false blossom. Phytopathology **35**: 805-821.
13. Kunkel, L. O. 1952. Transmission of alfalfa witch's broom to nonleguminous plants by dodder, and cure in periwinkle by heat. Phytopathology **42**: 27-31.
14. Maramorosch K. 1951. Handy insect-vector cage. Jour. N. Y. Entomol. Soc. 59: 49-50.
15. Raychaudhuri, S. P. 1952. Bayberry yellows. (Abs.) Phytopathology **42**: 17.
16. Severin, H. H. P. 1929. Yellows disease of celery, lettuce, and other plants, transmitted by Cicadula sexnotata (Fall.). Hilgardia **3**: 543-582.
17. Smith, K. M. 1937. A textbook of plant virus diseases. P. Blakiston's Son and Co., Inc., Phila. 615 pp.
18. Tomlinson, W. E., Jr., P. E. Marucci, and C. A. Doehlert. 1950. Leafhopper transmission of blueberry stunt disease. Jour. Econ. Entomol. **43**: 658-662.

FUNGI ASSOCIATED WITH THE DECAY OF WOODEN BUILDINGS IN NEW YORK STATE [1]

S. B. Silverborg [2]

Relatively few species of fungi are mentioned in the literature as being primarily responsible for decay of wood in buildings. In this country *Poria incrassata* (Berk. & Curt.) Burt, *Coniophora cerebella* Pers., *Fomes roseus* (Alb. & Schw. ex Fries) Cooke, and *Lenzites saepiaria* (Wulf. ex Fries) Fries, have been reported (7, 8, 9, 10, 12, 14) as the fungi most commonly associated with decay in wooden structures. In Europe Cartwright (1), Cartwright and Findlay (2), and others have listed *Merulius lacrymans* Wulf. ex Fries, *Poria vaillantii* (DC. ex Fries) Cooke, *Coniophora cerebella* and *Paxillus panuoides* Fries as the primary building decay fungi.

A knowledge of the specific fungi involved in decay of building timbers is important for decay control. These fungi not only have different requirements for their growth but vary considerably in tolerance to different wood preservatives. *Poria xantha* (Fries) Cooke, one of the fungi collected most frequently during this study, has been reported by Hirt (6) and Tippo et al. (15) to be tolerant of copper. Also, Duncan and Richards (5) in laboratory tests of various wood preservative have reported *Lenzites trabea* (Pers. ex Fries) Fries, another of the common species of building decay fungi, to be tolerant of pentachlorophenol.

From 1947 to 1951 a comprehensive survey was conducted in New York State to determine: the extent and underlying causes of wood decay in buildings (13); the construction methods leading to this defect; and the fungi commonly associated with the decay.

Survey methods.—Five hundred collections of decayed wood and sporophores were made from approximately 1500 buildings of various kinds in widely separated areas of New York State[3], during the spring, summer and fall of 1947 to 1951.

About 50 per cent of the total collections were obtained from urban and farm domiciles; the remaining 50 per cent were obtained from such structures as farm buildings other than houses, greenhouses, cold storage plants, icehouses, dairies and warehouses. Collections were obtained from buildings being remodeled or undergoing repair due primarily to decay damage, and from abandoned buildings. Decay data and fungous collections were obtained frequently from repair work brought to the attention of the writer by building contractors, lumber yard operators, and carpenters.

Identification of fungi.—The fungi were identified in most cases from sporophores associated with the decay. However, decay without sporophores was common, making it necessary to isolate the fungi from decayed wood and identify them by means of cultural characteristics. About 100 collections of decayed wood lacked fruit bodies of the causal fungus. When cul-

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[3] The area including New York City proper and its suburbs and Long Island Sound was not included in the survey.

TABLE 1.—*Fungi associated with decay of wood in buildings in New York State*

Fungi	Percent frequency of decay fungi according to type of structure						
	Total Percent Freq.	Urban and farm domiciles	Other farm buildings	Green-houses	Cold storage Plants & Icehouses	Dairies	Ware-houses
HYDNACEAE	19.0						
Odontia spathulata (Fries) Litsch.	12.8	6.8	3.4	1.0	0.4	0.6	0.6
Grandinia farinacea (Fries) Bourd. & Galzin	3.0	2.4	0.6				
Odontia crustosa (Fries) Quel.	1.2	0.8	0.4				
Steccherinum ochraceum (Fries) S. F. Gray	1.0		1.0				
Miscellaneous sp.[a]	1.0	0.6	0.4				
POLYPORACEAE	55.6						
Lenzites saepiaria (Wulf. ex Fries) Fries	12.4	4.8	3.2	3.0	0.6	0.4	0.4
Fomes roseus (Alb. & Schw. ex Fries) Cooke	7.6	4.8	0.8	0.6	0.6		0.8
Poria xantha (Fries) Cooke	6.2	2.6	0.8	1.4	0.4	0.4	0.6
Lenzites trabea (Pers. ex Fries) Fries	4.6	1.6	1.6	0.6		0.4	0.4
Poria ferruginosa (Schrad. ex Fries) Karst.	2.4	1.8	0.6				
Poria vaillantii (DC. ex Fries) Cooke	2.4	1.0	1.0	0.4			
Poria sinuascens Pilat	1.8	0.8	1.0				
Poria versipora (Pers.) Rom.	1.8	0.6	0.6		0.6		
Poria subacida (Peck) Sacc.	1.6		0.8		0.8		
Poria crassa (Karst.) Sacc.	1.6	0.6			1.0		
Poria crustulina Bres.	1.4		1.4				
Trametes mollis (Sommerf.) Fries	1.4	1.4					
Poria taxicola (Pers.) Bres.	1.2	1.2					
Poria sinuosa (Fries) Cooke	1.2	1.2					
Polyporus zonatus Fries	1.2					1.2	
Poria ambigua Bres.	1.2		1.2				
Miscellaneous sp.[a]	5.6	2.0	2.8		0.4		0.4
THELEPHORACEAE	21.6						
Peniophora pubera (Fries) Sacc.	2.6	1.6	1.0				
Corticium scutellare Berk. & Curt.	2.2	1.6	0.6				
Coniophora cerebella Pers.	2.0	1.0	1.0				
Undetermined sp.	14.8	6.8	3.8	1.0	1.2	1.2	0.8
AGARICACEAE	3.8						
Schizophyllum commune Fries	2.0		1.0		1.0		
Pholiota aeruginosa Peck	1.8	0.8	1.0				
Total	100.0	46.8	30.0	8.0	7.0	4.2	4.0

[a] Includes species for which only 1 collection was found.

tural identification was necessary, isolations were made on a 2 per cent malt-agar substratum. Keys on the cultural identification of fungi by Davidson et al. (4) and Nobles (11) were used to identify the fungi. The fungi isolated and identified by cultural characteristics were the same species as those identified from sporophore collections. Those isolated most frequently from decayed wood were *Lenzites saepiaria*, *Poria xantha*, and *Lenzites trabea*. Frequently it was impossible to obtain pure cultures of a fungus from decayed wood because of excessive mold or bacterial growth, or because the fungus had become inactive, especially in advanced stages of the brown rots.

RESULTS OF SURVEYS.—The species of fungi collected during this study are listed by families in decreasing order of occurrence in Table 1. The 2 species found most frequently were: *Odontia spathulata* (Fries) Litsch. (Fig. 1), one of the resupinate Hydnaceae, and *Lenzites saepiaria* of the Polyporaceae. They were about equally prevalent and composed 25 per cent of the total specimens. Other species of primary importance were *Fomes roseus*, *Poria xantha* and *Lenzites trabea*.

The resupinate Hydnaceae have been considered of minor importance in causing decay of wood in service; however, they comprised 19 per cent of the collections, consisting primarily of species of *Odontia*. A study of the taxonomy of the resupinate Hydnaceae and their relative importance in the decay of wood in New York was made by Czabator (3) in 1950.

The Thelephoraceae also were associated frequently with building decay. Twenty-one per cent of all the collections belonged to this group. The importance of the Thelephoraceae in the decay of building timbers is demonstrated in Table 1. While only 3 species are listed, constituting approximately 7 per cent of the total collection, an additional 14.8 per cent include undetermined species of *Peniophora*, *Coniophora*, and *Corticium*. There is need for a thorough taxonomic and decay study of this group of wood-rotting fungi.

Together the Hydnaceae and Thelephoraceae comprised approximately 40 per cent of all the decay fungi collected, which was unexpected since the Polyporaceae have been generally credited with causing most of the decay in wooden buildings.

Among the fungi found less commonly but frequent-

FIG. 1.—Portion of fruiting surface of *Odontia spathulata* from decayed roof timber of a house. The flattened confluent teeth, fimbriate or divided at the apices, are characteristic features of this fungus (×9).

ly enough to be considered among the important species were *Grandinia farinacea* (Fries) Bourd. & Galzin of the resupinate Hydnaceae; *Peniophora pubera* (Fries) Sacc., *Corticium scutellare* Berk. & Curt., and *Coniophora cerebella* of the Thelephoraceae; *Poria ferruginosa* (Schrad. ex Fries) Karst. and *Poria vaillantii* of the Polyporaceae; and *Schizophyllum commune* Fries of the Agaricaceae.

Merulius lacrymans, the dry-rot or tear fungus, one of the most destructive building decay fungi in Europe, is rare in this country. However, 1 collection was found in northern New York during this investigation.

The rot produced by the common species of building decay fungi is a brown cubical type with the exception of a white stringy rot associated with *O. spathulata*.

SUMMARY

Twenty-five species of fungi, and several unidentified species of Thelephoraceae were found associated with decay of wood in buildings in New York State.

Odontia spathulata and *Lenzites saepiaria* were found most frequently and comprised approximately 25 per cent of the collections. Other species of primary importance were *Fomes roseus*, *Poria xantha*, and *Lenzites trabea*.

Other important species were *Grandinia farinacea*, *Peniophora pubera*, *Corticium scutellare*, *Schizophyllum commune*, *Poria ferruginosa*, *Coniophora cerebella* and *Poria vaillantii*.

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LITERATURE CITED

1. CARTWRIGHT, K. ST. G. 1935. Decay of timber in houses. Chartered Surveyors' Institution, London. Paper 561.
2. CARTWRIGHT, K. ST. G., AND W. P. K. FINDLAY. 1945. Dry rot in wood. Bul. For. Prod. Res., London, No. 1. 4th Ed.
3. CZABATOR, F. J. 1950. The resupinate hydnaceae of New York and their relation to the decay of wood. State University of New York, College of Forestry (unpublished thesis).
4. DAVIDSON, R. W., W. A. CAMPBELL, AND DOROTHY J. BLAISDELL. 1938. Differentiation of wood-decaying fungi by their reactions on gallic or tannic acid medium. Jour. Agri. Res. [U. S.] **57**: 683-695.
5. DUNCAN, CATHERINE G. AND C. AUDREY RICHARDS. 1948. Methods for evaluating wood preservatives: weathered impregnated wood blocks. Proc. Amer. Wood-Preservers' Association **44**: 259-268.
6. HIRT, R. R. 1949. An isolate of poria xantha on media containing copper. Phytopathology **34**: 31-36.
7. HOXIE, F. J. 1930. Decay of wood in industrial buildings. Associated Fac. Mut. Fire Insur. Co., Boston.
8. HUBERT, E. E. 1929. The cause and control of decay in buildings. University of Idaho Bul. 2.
9. HUMPHREY, C. J. 1923. Decay of lumber and building timbers due to Poria incrassata. Mycologia **15**: 258-277.
10. HUMPHREY, C. J., AND L. E. MILES. 1925. Dry-rot in buildings and stored construction materials and how to combat it. Ala. Polytech. Inst. Circular 78.
11. NOBLES, MILDRED K. 1948. Studies in forest pathology, VI. Identification of cultures of wood-rotting fungi. Can. Jour. Res. C. **26**: 281-431.
12. RICHARDS, C. AUDREY. 1933. Decay in buildings. Proc. Amer. Wood Pres. Assoc. 389-398.
13. SILVERBORG, S. B. 1951. Wood decay in houses. State Univ. of New York. College of Forestry Circ. Vol. 24. No. 1.
14. SNELL, W. H. 1922. Studies of certain fungi of economic importance in the decay of building timbers. U. S. Dept. Agri. Bul. 1053.
15. TIPPO, O., J. M. WALTERS, S. J. SMUCKER, AND W. SPAKMAN, JR. 1947. The effectiveness of certain wood preservatives in preventing the spread of decay in wooden ships. Lloydia **10**: 175-208.

PARTIAL PURIFICATION AND PROPERTIES OF THE ANTIFUNGAL ANTIBIOTIC, TOXIMYCIN [1]

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A series of papers from this laboratory has reported studies on antibiotics in relation to plant disease problems (4, 5, 6, 7, 8, 9, 10, 14). Two new antifungal antibiotics, antimycin and helixin, have been reported; the present paper deals with a third, which has been tentatively designated toximycin. Work on the production, assay, purification, and properties of this material is reported. A brief account of some of the work has already appeared (13).

Toximycin is produced by *Bacillus subtilis* Cohn *emend.* Prazmowski, our culture No. A67. A detailed description of this organism is given elsewhere (12). An earlier study indicated that the antibiotic produced by *B. subtilis* A67 inhibited all of the 8 phytopathogenic fungi and 2 phytopathogenic bacteria tested, and that the antibiotic was comparatively stable and could be produced in liquid media (14).

PRODUCTION AND ASSAY.—In previous studies it was found that an antibiotic was produced by *B. subtilis* A67 when the organism was grown in several liquid media (14). One of these, medium B, was used in the present studies. Four hundred ml. amounts of the medium and a pellet of DC Antifoam A (Dow Corning Corp., Midland, Michigan) 0.5 cm. in diameter were added to 2 l.Erlenmeyer flasks, which were autoclaved ½ hour at 15 psi. Inoculum for each flask consisted of 20 ml. of a water suspension of cells of the antibiotic organism prepared from yeast extract-glucose agar[2] slants that had been kept at 28° C. for 48 hours. Flasks were incubated at 26° on a reciprocal shaker making 66 cycles per minute with a 15 cm. stroke. A maximum antibiotic concentration of 2 to 3 units (a unit is defined below) per ml. of culture centrifugate was obtained in 4 days, and the pH change during this time was usually from 7.0-7.2 to 8.5-8.8.

A cylinder plate diffusion assay was used for the estimation of toximycin. Ten ml. lots of assay agar (per l.: peptone, 5 gm.; agar, 17 gm.) were added to Petri plates. Three or 4 "penicylinders" were placed equidistant on the hardened agar and 5 ml. of assay agar seeded with *Glomerella cingulata* (Stonem.) Spauld. and v. Schrenk was added to each plate. The method of making the seed layer has been indicated elsewhere (1). To evaluate the potency of a given preparation, 9 cylinders on 3 assay plates were used. After an incubation period of 36 to 40 hours at 26°, the average inhibition zone size was determined. The potency of a given preparation, expressed in "units," was calculated by reference to a dosage-response curve drawn on the basis of the inhibition zones produced by a standard preparation that was tested at different concentrations each time an assay was made. A partly purified preparation made according to the procedure indicated in Table 1 was used as a standard. This material, which appeared to have retained all of its potency over a 6 month period, was arbitrarily assigned a potency of 8 units per mg. and was tested at 1, 2, 4, and 8 units per ml. in 0.05M potassium phosphate buffer at pH 6.8. Each concentration of the standard was tested in 1 penicylinder in each of 3 plates. One unit of activity usually produced inhibition zones 21–22 mm. in diameter.

Zone diameter of the standard was found to be a straight line function of the log of the antibiotic concentration, and dosage-response curves of culture centrifugates when diluted with water or with potassium phosphate buffer[3], and of partly purified fractions that were diluted in the same manner, usually were straight lines parallel to the standard. Inhibition zones were free from growth of the assay organism in the central area; toward the circumference of the zone, however, there was a limited area of sparse growth, usually 1 mm. wide. Measurements included this incompletely inhibited area.

FRACTIONATION AND PROPERTIES.—Early studies on the fractionation of the antibiotic material in the *B. subtilis* A67 culture centrifugates showed that all or nearly all of the antibiotic was extractable with *n*-butanol. In later studies it was found more convenient to recover the active material from the precipitate that formed when the culture centrifugate was acidified. This method was used to obtain "partly purified toximycin" preparations used in the remainder of the work; an example fractionation is given in Table 1. Yields of 121 to 249 mg. per l., containing 4-8 units per mg., were obtained. The final products represented a 34- to 65-fold purification and a recovery of approximately ½ the original activity.

Solubility, stability, and other tests were made with a toximycin preparation (prepared as in Table 1) containing 4 units per mg. To test solubility, 16 mg. quantities of the preparation were added to 2 ml. of various organic solvents, and 4 mg. quantities were added to 2 ml. amounts of water and adjusted to 8 pH levels. If undissolved material was present after 2

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[2] Per l.: Bacto yeast extract, 2 gm.; glucose, 10 gm.; agar, 17 gm.

[3] Throughout the remainder of the paper, except in the section dealing with the tobacco necrosis virus, the term "buffer" refers to 0.05M potassium phosphate at pH 6.8.

TABLE 1.—*Procedure used for obtaining partly purified toximycin*

Step	Treatment	Fraction tested	Volume or wt.	Total activity in units	Units per mg. dry wt.
1.	Eleven l. of pooled cultures made to pH 9 and centrifuged[a]. Residue discarded.	Centrifugate	10 l.	20,000	0.12
2.	pH of centrifugate changed to 2.5 (HCl). Suspension filtered with aid of Celite 503.	----	----	----	----
3.	Residue extracted successively with 2 1-l. and 1 0.5-l. amounts of ethanol. Residue discarded.	----	----	----	----
4.	Ethanol extract taken to 500 ml. under reduced pressure. Two l. of diethyl ether added. Precipitate recovered by decanting and centrifuging.	Supernatant	----	<100	----
5.	Precipitate dried over P_2O_5. "Partly purified toximycin."	Precipitate	1.217 gm.	9,736	8.0

[a] It was found that centrifugation (Sharples Super Centrifuge, about 50,000 x g.) was more satisfactory if the cultures were first made to pH 9.0.

hours, the preparation was filtered through a fine filter paper, and the residue dissolved in the buffer and assayed. The aqueous filtrates were adjusted to pH 7, made to 4 ml., and assayed. On the basis of assays, the antibiotic was indicated to be soluble in water at pH 6 to 10, 80 per cent ethanol, 50 per cent acetone; less soluble in water at pH 5 to 6, absolute ethanol, and glacial acetic acid; slightly or slowly soluble in dioxan, *n-butanol*, and isopropanol; and insoluble in acetone, ether, chloroform, benzene, and methyl, ethyl, and amyl acetates, and in water more acidic than pH 5. When the partly purified material was added to distilled water, the resulting suspension had a pH value of 4.6; the suspended particles could be brought into solution by adjusting the pH to 7.

Stability at 100° was tested at pH 2, 7, and 10 in aqueous preparations and in the buffer; after 30 minutes at 100° the approximate activity remaining in the solutions was found to be, respectively, 50, 46, 0, and 88 per cent of the original. When the antibiotic in the buffer solution was autoclaved for 15 minutes at 10 psi, no loss in activity could be detected.

Aqueous suspensions of the partly purified material gave positive Millon, xanthoproteic, biuret, and ninhydrin tests, indicating that material of a polypeptide or protein nature was present. The Fehling's test was negative. As determined by assay, the antibiotic was slightly or slowly dialyzable, and was not inactivated by pepsin or trypsin.

BIOLOGICAL STUDIES.—Studies on the biological properties of partly purified toximycin were of 4 types: agar-streak tests, tobacco necrosis virus inhibition tests, early blight protectant tests, and tests for phytotoxicity. A partly purified toximycin preparation containing 4 units per mg. was used in all these experiments, except in the agar-streak tests, where a preparation containing 8 units per mg. was used.

Agar-streak tests.—The effect of toximycin on a number of microorganisms was examined by means of agar-streak tests in 2 trials. In these tests various concentrations of the partly purified material in the buffer were autoclaved for 15 minutes at 10 psi and mixed in warm agar media, which were allowed to harden. Media containing the antibiotic, as well as suitable control media, were then streaked with spore or cell suspensions of test organisms. The addition of the antibiotic did not change the pH of the media. After suitable incubation periods the inhibition point for each organism was noted. Suspensions for seeding fungi, except *Streptomyces scabies* (Thaxt.) Waks. and Henrici, *Mycobacterium avium* Chester, and *M. phlei* Lehmann and Neumann, were prepared from cultures that were grown on potato-glucose agar[4] at 24° for 7 to 14 days. Those for *M. avium* were prepared from cultures that were grown on potato-glucose agar at 36° for 7 days. Cultures for making suspensions of *M. phlei*, *S. scabies*, and of all bacteria, except the *Corynebacterium* species, were prepared from nutrient agar[5] cultures that had grown at 24° or 36° for 24 to 72 hours. Suspensions of the *Corynebacterium* species were prepared from meat infusion agar[6] cultures that had grown at 36° for 48 hours.

Data obtained in the agar-streak tests, the media used, and conditions of incubation for the test organisms that were inhibited are in Table 2. Most of the fungi were inhibited completely by concentrations of 25 μg. per ml. or less, whereas most of the bacteria were not inhibited by 100 μg. per ml. The names of organisms that were not inhibited in either test by 100 μg. per ml. follow: *Agrobacterium tumefaciens* (Smith and Towns.) Conn, *Erwinia amylovora* (Burr.) Winslow *et al.*, *Escherichia coli* (Migula) Castellani and Chalmers, *M. avium*, *M. phlei*, *Pseudomonas aeruginosa* (Schroeter) Migula, *Pythium debaryanum* Hesse, *P. ultimum* Trow, *Xanthomonas phaseoli*

[4] Per l.: extract from 200 gm. of sliced, peeled potatoes steamed ½ hour in 500 ml. of distilled water; glucose, 25 gm.; agar, 17 gm.

[5] Per l.: Peptone, 10 gm.; beef extract, 3 gm.; glucose, 10 gm.; agar, 20 gm.

[6] Meat infusion broth (2) with 17 gm. of agar per l. added.

TABLE 2.—*Inhibition of various microorganisms by partly purified toximycin. Agar-streak tests*

Microorganism	Medium[a]	Incubation		Inhibition point[b]
		Time	Temp.	
		hrs.	°C.	μg. per ml.
Alternaria solani (E. and M.) Jones and Grout	PA	96	24	25
Bacillus cereus var. *mycoides* (Flügge) Bergey et al.	NA	48	36	25, 50
Botrytis allii Munn	PA	96	24	25
Chalara quercina Henry	PA	96	24	25
Colletotrichum circinans (Berk.) Vogl.	PA	96	24	12.5, 25
C. phomoides (Sacc.) Chester	PA	96	24	12.5, 25
Corynebacterium sp.[c]	MI	48	36	250
Fusarium oxysporum f. *lycopersici* (Sacc.) S. and H.	PA	96	24	50, 100
F. oxysporum f. *pisi* (Linford) S. and H.	PA	96	24	50
Gliocladium fimbriatum Gilman and Abbott	PA	96	24	25
Glomerella cingulata (Stonem.) Spauld. and v. Schrenk	PA	96	24	12.5, 25
Helminthosporium sativum Pam., King, and Bakke	PA	96	24	25
H. victoriae M. and M.	PA	96	24	25, 50
Micrococcus pyogenes var. *aureus* (Rosenbach) Zopf	NA	48	36	50, 100
Nigrospora sphaerica (Sacc.) Mason	PA	96	24	6.25
Penicillium notatum Westling	PA	96	24	25
Pellicularia filamentosa (Pat.) Rogers	PA	72	24	25, 50
Saccharomyces cerevisiae Meyen	PA	96	24	50
Sclerotinia fructicola (Wint.) Rehm	PA	96	24	25
Stemphylium sarcinaeforme (Cav.) Wilts.	PA	96	24	25, 50
Streptomyces scabies (Thaxt.) Waks. and Henrici	NA	48	36	> 100, 100
Trichophyton mentagrophytes (Robin) Blanchard	PA	96	24	25
Venturia inaequalis (Cke.) Wint.	PA	192	24	6.25
Verticillium albo-atrum Reinke and Berth.	PA	96	24	25, 50
Xanthomonas campestris (Pammel) Dowson	NA	48	24	25, 50

[a] NA: Nutrient agar (footnote 5); pH 6.2–6.4. PA: Potato agar, prepared as follows: per 1.: extract from 40 gm. of sliced, peeled potatoes steamed ½ hour in 500 ml. water; glucose, 5 gm.; agar, 17 gm.; pH 5.9. MI: Meat infusion agar (footnote 6); pH 7.0.

[b] The smallest amount (dry weight) that completely inhibited macroscopic growth. Two figures are given where the inhibition points differed in the 2 tests.

[c] Diphtheroid culture; does not produce diphtheria toxin.

(Smith) Dowson, and *X. translucens* f. sp. *cerealis* Hagborg.

Tobacco necrosis virus inhibition tests.—The ability of toximycin to inhibit the growth of certain bacteria and fungi suggested the advisability of testing the effect of this material *in vitro* on plant viruses, and the tobacco necrosis virus was chosen for these studies. A suspension of ground, dehydrated leaves of *Nicotiana tabacum* L. infected with the virus, strain "RR"[7], was prepared in 0.1 M sodium phosphate buffer at pH 7, using approximately 0.4 mg. of ground leaves per ml. Equal parts of the suspension and of solutions of a toximycin preparation in the same buffer were mixed and allowed to stand at 22–24° for about 1 hour. Suitable control solutions containing the antibiotic or the virus suspension were also made. The primary leaves of cowpea plants grown in the greenhouse were inoculated after the manner of Leben and Fulton (5), and after an incubation period of 3–6 days the number of lesions was counted. There were 3 tests, each using 4 concentrations of the antibiotic preparation, and at each concentration 6 leaves were inoculated.

In all 3 tests the antibiotic preparation inhibited lesion production. At 2 mg. per ml. a total of 28 lesions was found on the test leaf halves as compared with 296 lesions on the control leaf halves. At 1 mg. per ml. the numbers were 24 and 354. At 0.5 or 0.25 mg. per ml. the degree of inhibition was not so marked.

[7] We wish to thank Dr. R. W. Fulton for this material.

Early blight protectant tests.—Partly purified toximycin was tested as a protectant fungicide in the greenhouse against the early blight disease on tomato (*Lycopersicon esculentum* Mill.) foliage, incited by *Alternaria solani*.

In general, the methods of Leben and Keitt (7) were used. Test sprays contained different quantities of the antibiotic in distilled water; these preparations were adjusted to pH 7 with NaOH. Four plants were used for each concentration of the antibiotic and for the control spray in each of 3 trials. Plants were 15–20 cm. high. Lesion counts were made on the third, fourth, and fifth leaves above the cotyledons, and an average count for each treatment was determined. The average ED (effective dose) 50 and ED 95 for the 3 tests were found to be 44 (range: 23 to 68) and 300 (range: 195 to 480) μg. per ml., respectively.

Tests for phytotoxicity.—In these tests toximycin solutions were applied to the foliage of various plants and were used in tests with tomato cuttings. A brief account of these experiments follows. In the foliage tests, of which 2 were made, the antibiotic at pH 7 was sprayed on the leaves of young tomato, tobacco, bean (*Phaseolus vulgaris* L.), cowpea (*Vigna sinensis* (L.) Endl.), and cucumber (*Cucumis sativus* L.) plants. The antibiotic concentrations used were 0.5 and 1.0

mg. per ml. No injury to the plants was observed up to 14 days, when the experiment was concluded.

The effect of a toximycin preparation was further studied by immersing stems of tomato cuttings, approximately 25 cm. long, in different concentrations of the antibiotic at pH 7 for 2 days. Two tests were made, and in each, 2 cuttings were used at each of 6 concentrations of antibiotic in 10 ml.; the concentrations were from 0.25 to 8 units per ml. Water was used as a control. At the end of 2 days the volume of solution taken up by cuttings was noted, and cuttings transferred to tubes of water. Antibiotic solutions not taken up were made to 10 ml. and assayed. A loss in antibiotic activity was considered to be the result of uptake by cuttings, because no activity was lost in antibiotic solutions that did not contain cuttings. In both tests, 2.5 units per cutting was taken up without apparent injury, but when greater amounts were taken up, wilting occurred. Cuttings in the most concentrated solution took up an average of 58.5 and 70 units per cutting in the 2 tests, which represented 72 and 88 per cent of the antibiotic available. Wilted cuttings recovered completely within 5 days after transfer to distilled water. There was a decrease in water uptake and a marked stimulation in number and length of rootlets that formed on stems of cuttings that had been in the antibiotic solutions and had been transferred to water.

Discussion.—A partly purified antibiotic that inhibited the growth of a number of fungi and some bacteria is described. This material appeared to have considerable potency when tested *in vitro* against fungi and as a protectant fungicide in the greenhouse, and it also inhibited a plant virus *in vitro*, as have certain other substances obtained from microorganisms (e.g., 3, 15).

The name toximycin was proposed (13) for the antibiotic material described in this paper after a survey of the properties of 22 antibiotics that have been reported to be produced by *B. subtilis* or synonymous species. Like toximycin, most of these materials are classed as polypeptides. However, they differ from toximycin in one or more physical, chemical, or biological properties. It is our feeling that the name toximycin must be provisional, however, since in the study of the *B. subtilis* antibiotics, different testing procedures and various organisms or lines of a given organism have been used in inhibition experiments. Also, properties of some materials have not yet been reported in full. The name toximycin was suggested by the marked inhibitory effect of the antibiotic on a number of fungi.

SUMMARY

An antibiotic, tentatively designated toximycin, was partly purified by fractionation of shaken cultures of a *Bacillus subtilis* isolate obtained by the writers from soil. The *Glomerella cingulata* agar diffusion assay was used to estimate activity. A recovery of about ½ of the active material with a 34- to 65-fold purification was obtained.

Properties of the antibiotic in the partly purified form are reported. Polypeptide tests were positive. Twenty fungi and 3 bacteria were completely inhibited at concentrations from 6.25 to 100 μg. per ml. in agar-streak tests, and in *in vitro* tests the tobacco necrosis virus was inhibited. In protectant fungicide studies in the greenhouse with *Alternaria solani* on tomato foliage, the ED 50 and ED 95 were found to be 44 and 300 μg. per ml., respectively. Tomato cuttings in solutions of the antibiotic took up a maximum of 58.5 to 70 units per cutting; an uptake greater than 2.5 units resulted in wilting. Wilted cuttings recovered on transfers to water, but subsequent water uptake was reduced. The partly purified material was nontoxic to 5 species of plants when applied as a spray.

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LITERATURE CITED

1. Dunshee, B. R., C. Leben, G. W. Keitt, and F. M. Strong. 1949. The isolation and properties of antimycin A. Jour. Amer. Chem. Soc. **71**: 2436-2437.
2. Frobisher, Jr., M. 1944. Fundamentals of bacteriology. 3rd ed. W. B. Saunders Co. Philadelphia and London. 824 pp.
3. Gupta, B. M., and W. C. Price. 1950. Production of plant virus inhibitors by fungi. Phytopathology **40**: 642-652.
4. Leben, C., and D. C. Arny. 1952. Seed treatment experiments with helixin B. (Abs.) Phytopathology **42**: 469.
5. Leben, C., and R. W. Fulton. 1952. Effect of certain antibiotics on lesion production by two plant viruses. Phytopathology **42**: 331-335.
6. Leben, C., and G. W. Keitt. 1948. An antibiotic substance active against certain phytopathogens. Phytopathology **38**: 899-906.
7. Leben, C., and G. W. Keitt. 1949. Laboratory and greenhouse studies of antimycin preparations as protectant fungicides. Phytopathology **39**: 529-540.
8. Leben, C., and G. W. Keitt. 1952. Studies on helixin in relation to plant disease control. Phytopathology **42**: 168-170.
9. Leben, C., G. J. Stessel, and G. W. Keitt. 1952. Helixin, an antibiotic active against certain fungi and bacteria. Mycologia **44**: 159-169.
10. Smeby, R. R., C. Leben, G. W. Keitt, and F. M. Strong. 1952. Production and purification of the antibiotic helixin. Phytopathology **42**: 506-510.
11. Stessel, G. J. 1951. Methods of selecting soil microorganisms that produce antibiotics active against plant pathogens. (Abs.) Phytopathology **41**: 33.
12. Stessel, G. J. 1952. A search for antibiotics for use in plant disease control. Thesis, library of the University of Wisconsin, Madison, Wisconsin.
13. Stessel, G. J., C. Leben, and G. W. Keitt. 1952. Studies on an antifungal antibiotic produced by Bacillus subtilis. (Abs.) Phytopathology **42**: 20.
14. Stessel, G. J., C. Leben, and G. W. Keitt. Screening tests designed to discover antibiotics suitable for plant disease control. Mycologia (In press.)
15. Utech, N. M., and J. Johnson. 1950. The inactivation of plant viruses by substances obtained from bacteria and fungi. Phytopathology **40**: 247-265.

FACTORS AFFECTING PLAQUE FORMATION BY XANTHOMONAS PRUNI BACTERIOPHAGE [1]

J. D. Mandell [2] and A. Eisenstark [3]

At least 5 distinct types of *Xanthomonas pruni* bacteriophage have been differentiated on the basis of plaque morphology (7). In addition, different host strains may display different plaque types when attacked by the same variety of phage. Since the observation of lysis on the surface of an agar plate is a key in the analysis of phage-host actions, it was desirable to determine the factors that cause variation in plaque morphology.

METHODS AND MATERIALS.—The bacterial host was an American Type Culture No. 10016 and was routinely grown in standard peptone-beef extract medium to which 0.5 per cent sodium chloride was added, for reasons described below. All plating for plaque production was done by a 2-layer method in which 4.5 ml. of broth containing virus and bacteria were mixed with 1.5 ml. of melted 2 per cent nutrient agar and poured over a layer of solid nutrient medium. Plates were incubated at 27° C. for 1–2 days unless otherwise stated.

RESULTS.—To determine the effect of age of the plating bacterial culture on plaque size, a broth culture was inoculated from a stock slant; samples taken over a period of 22 hours were plated with an appropriate dilution of phage stocks. After plates had incubated for 3 days, 100 plaques from each age sample were measured optically with a stage micrometer to determine the mean diameter. Figure 1 shows the decrease in plaque size with older samples of host culture.

Plates were prepared with different concentrations of agar to observe the effect on plaque morphology. Concentrations of 0.5, 1.0, 1.25, 1.5 and 2.0 per cent were used in the agar surface layer. It was found that increased agar concentration decreased the size and distinctness of plaques, but the effect was not so pronounced as the other factors described.

The effect of increased sodium chloride concentration in the plating medium—top and bottom layers having the same concentrations—was the increase of plaque size to a maximum at the highest concentration of sodium chloride in which the host would grow. The increased sodium chloride concentration also increased the halo size, thus radically changing the appearance of the plaque. Figures 2A and 2B illustrate the difference in size and appearance of plaques produced on 0.5 and 3.0 per cent sodium chloride, respectively.

When different amounts of the same turbid host culture were plated, the size of plaques was inversely proportional to the amount of host cells plated, as may be noted from figures 2C and 2D. This may explain the results shown in figure 1; the differences in plaques may be due to differences in numbers of host cells, rather than differences in age of culture.

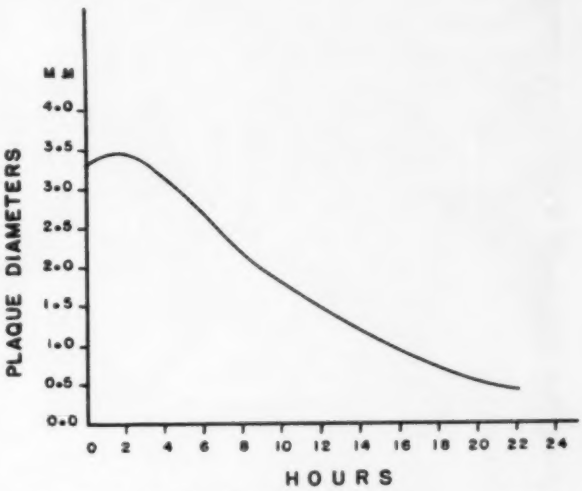

FIG. 1. Graph of relation of plaque size to age of host culture.

Higher incubation temperature was found to decrease plaque size, as illustrated in Figures 2E and 2F. Temperature increases above 25° C. resulted in smaller plaques; at 30° C. plaques were minute and irregular; above 33° C. plaque formation was not observed, even though the host grew well at this temperature.

DISCUSSION.—The size of plaques was observed to decrease with increased inoculum or upon use of an older bacterial culture, an older culture probably being in effect merely an increased inoculum. This phenomenon of decreased plaque size with increased number of plating bacteria is well known and appears to be true for several phages (2, 6).

Increased plaque size with increased salt concentration has been demonstrated by Hershey, et al (4), who showed that salt was necessary before adsorption of virus to host cell would take place. While this may also be the case for pruniphage, another pertinent fact should be mentioned. *X. pruni* cells take up oxygen at a slower rate in 3.0 per cent sodium chloride than in 0.5 per cent and produce more cells in the lower salt concentration in a given length of time[4]. If this high salt concentration slowed the growth of the bacteria more than it affected the rate of replication of the virus, larger plaques should be expected, based upon the results in Figure 1. On the contrary, smaller

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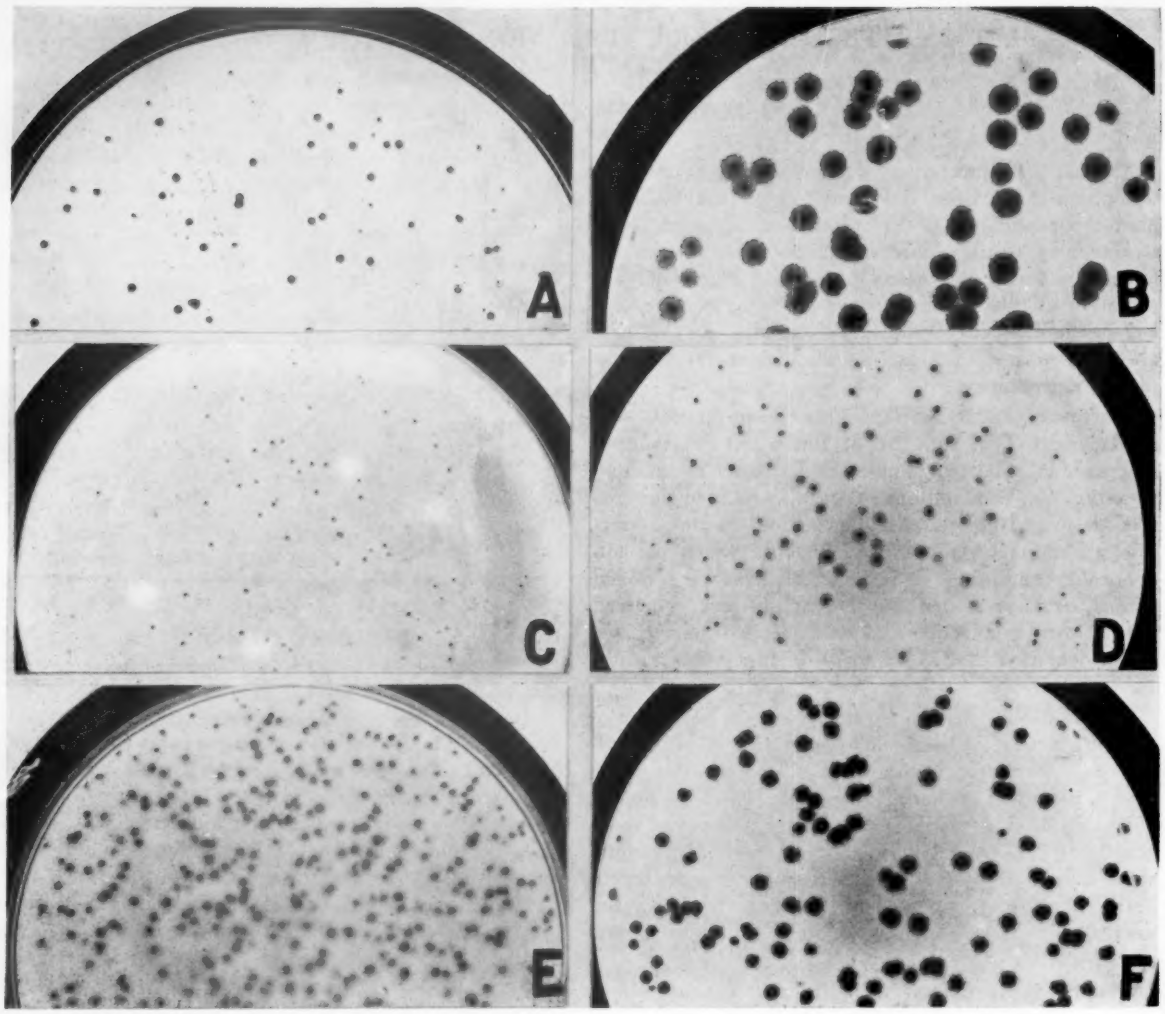

FIG. 2. Photographs of phage plaques on petri plates. A) 0.5 per cent sodium chloride in medium. B) 3.0 per cent sodium chloride in medium. Considerably larger plaques are produced with the higher salt concentration. C) 0.5 ml. of turbid host suspension. D) 0.05 ml. of turbid host suspension. The more dilute suspension results in larger plaques with more distinct halo formation. E) Plates incubated at 30° C. F) Plates incubated at 25° C. Plaques are much larger at the lower temperature.

Photographs in Figure 2 were prepared by placing petri dishes directly into the photographic enlarger and using the plates as negatives. The plaques were then projected on sensitive paper. This method was particularly useful as a routine for recording plaques during experiments. The method is speedy, and no camera is needed.

plaques actually result.

Observations on the effect of temperature on plaques produced by pruniphage agree with those of other workers (1, 6). The reason for the diminished size of plaques with increased temperature and lack of plaque formation above 33° C. is obscure, since it is well below the inactivation temperature of pruniphage (3), and the host organism grows well at this temperature. A possible explanation may lie in the increased growth rate of the host at higher temperatures, since plaque formation has been reported to be a function of division of the uninfected cells (1).

The above information is useful for additional reasons. For example, recent studies (5, 8) have promised the development of phage typing methods for the identification of plant pathogens, methods which have been valuable in the identification of *Salmonella*, *Micrococci*, *Shigella* and other bacteria. Such methods for the identification or typing of *X. pruni* strains would yield unreliable results unless care were taken to add salt to the medium and to incubate plates at a proper temperature, several degrees below the maximum temperature for the growth of the host, *X. pruni*.

SUMMARY

Plaques produced by *X. pruni* bacteriophage decrease in size with increased concentration of plating bacteria, increase in size in the presence of sodium chloride in the plating medium in the highest concen-

tration permitting growth of the host, and decrease in size with incubation temperatures above 27° C. Plaque formation was not observed above 33° C., although this temperature is well below the inactivation temperature of the pruniphage and well below the temperature at which the host will grow. This information is useful in the analysis of pruniphage-host action and is necessary for the development of an adequate phage-typing method for the identification of plant pathogenic bacteria.

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LITERATURE CITED

1. ANDERSON, T. F. 1948. The influence of temperature and nutrients on plaque formation by bacteriophages active on E. coli strain B. Jour. Bact., **55**: 659-665.
2. CHERRY, W. B. AND D. W. WATSON. 1949. The Streptococcus lactis host-virus system. I. Factors influencing quantitative measurement of the virus. Jour. Bact., **58**: 601-610.
3. CROWNOVER, RUTH, J. D. MANDELL AND A. EISENSTARK. 1950. Studies of a bacterial virus active against Xanthomonas pruni. Proc. Okla. Acad. Sci., **31**: 40-42.
4. HERSHEY, A. D. AND J. BRONFENBRENNER. 1945. Transmissible variation of plaque-type in bacteriophage. Jour. Bact., **49**: 107.
5. KATZNELSON, H. AND M. D. SUTTON. 1951. A rapid phage plaque count method for the detection of bacteria as applied to the demonstration of internally borne bacterial infections of seed. Jour. Bact., **61**: 689-701.
6. KLECZKOWSKA, J. 1945. A quantitative study of the interaction of bacteriophage with Rhizobium, using the technique of poured plates. Jour. Bact., **50**: 81-94.
7. MANDELL, J. D. AND A. EISENSTARK. 1952. Bacterial viruses active against Xanthomonas pruni. Differentiation of genetic types. Monthly Research Rept., Office Naval Research, April issue, 1-4.
8. THORNBERRY, H. H., A. C. BRAUN, AND R. P. ELROD. 1949. Application of the bacteriophage-lysis technique for the identification of plant pathogenic bacteria. Phytopathology, **39**: 152-154.

ACQUISITION THRESHOLD PERIOD OF WESTERN CELERY MOSAIC VIRUS FOR FOUR SPECIES OF APHIDS [1]

John N. Simons [2] and Edward S. Sylvester [2]

Watson and Roberts (22) listed as one criterion of the nonpersistent group of aphid-borne viruses their capacity to be acquired by their vectors in short feeding times (less than 2 minutes). Several viruses of this group have had this short acquisition threshold period demonstrated. These include nasturtium mosaic (5), beet mosaic (17, 19), *Brassica nigra* virus (18), and alfalfa mosaic (14). Although the western celery mosaic virus had been shown by Severin and Freitag (11) to be readily juice inoculable and to be retained for less than 24 hours in its aphid vectors—facts which definitely indicated that this virus was another of the nonpersistent type—it was felt desirable to determine tne acquisition threshold period in order to characterize it more accurately as belonging to this group.

DEFINITION OF TERMS.—During the last decade a number of terms defining various vector-virus relationships have been proposed; several are synonymous. Since only one term is necessary to designate any particular vector-virus relationship, the authors propose the use of the following terms.

Acquisition threshold period.—Sylvester (16) used the term "acquisition threshold" to denote the minimum time necessary for a vector to feed upon a virus source in order to obtain an infective charge. Prentice and Woollcombe (10) correctly pointed out that the word "threshold" does not usually imply duration and proposed the addition of the word "period."

Acquisition feeding or acquisition feeding period.—Several terms are found in the current literature which refer to the time a noninfective vector feeds upon a virus source. These include "infection feeding (period)" (2, 3, 7, 9, 10, 12, 13, 15, 16, 21), "infective feeding" (6), "acquisition feed" (8), and "acquisition feeding (period)" (1, 4, 5). Of these the authors suggest that "acquisition feeding (period)" is the most self-explanatory.

Test feeding or test feeding period.—Among the terms used to designate the time during which a vector is fed upon a healthy test plant are "test feeding (period)" (4, 7, 13, 16), "inoculation feeding (period)" (1, 3), and "transfer feed" (8, 10). Of these "test feeding (period)" seems to be the most common in usage and the authors propose to continue its usage.

MATERIALS AND METHODS—The western celery mosaic virus was obtained from infected greenhouse celery. Four species of aphids were used in the tests: the green peach aphid, *Myzus persicae* (Sulz.); the foxglove aphid, *M. solani* (Kalt.); the crescent marked lily aphid, *M. circumflexus* (Buckt.); and the celery aphid, *Aphis apii* Theob. Noninfectives of the green peach aphid were reared on smooth-leafed mustard, *Brassica juncea* Czern. et Cross. The other 3 species were maintained on celery. All insect transfers were

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made with a camel's-hair brush and feedings were timed with a stop-watch. Fasting was carried out in glass vials. Young celery seedlings were used as test plants. Thirty replications of each treatment, 1 plant per treatment, were done and the use of any particular aphid species in each replication was determined by randomization. After the test feeding period the plants were fumigated with nicotine and placed in the greenhouse for incubation.

RESULTS.—To determine the acquisition threshold period, the following procedure was used. The 4 starvation period of 30-91 minutes. ($\bar{x}$ = 75.04 min.),[3] species of aphids were given acquisition feedings of 5, 10, 15, 20, 25, and 30 seconds after a preliminary The ranges (means) of the acquisition feeding periods for the 4 aphid species were as follows: green peach aphid 5(5.0) seconds, 10(10.0) seconds, 14-16(15.0) seconds, 19-21(20.0) seconds, 24-25(24.8) seconds, and 29-30(29.9) seconds; foxglove aphid 5(5.0) seconds, 10(10.0) seconds, 15-16(15.1) seconds, 19-21(19.9) seconds, 24-25(24.8) seconds, and 29-30(30.0) seconds; crescent marked lily aphid 5(5.0) seconds, 10-11(10.1) seconds, 14-16(15.1) seconds, 19-20(19.9) seconds, 24-26(25.0) seconds, and 29-30(29.9) seconds; celery aphid 5(5.0) seconds, 10(10.0) seconds, 15(15.0) seconds, 20-21(20.1) seconds, 24-26(25.0) seconds, and 29-31(30.0) seconds. Results of the trials are in Table 1. None of the species was able to acquire sufficient virus in 5 seconds feeding to infect a plant, but all 4 species successfully acquired virus in the 10 second interval. The celery aphid was the most efficient vector; the foxglove aphid was intermediate and the other 2 species were both relatively inefficient. There seemed to be little difference in the method in which the virus was acquired by any of the 4 species.

DISCUSSION.—Severin and Freitag (11), in their work on western celery mosaic virus, used all 4 of these aphid species as experimental vectors. When a leaf transfer technique was employed the order of efficiency of transmission was: celery aphid 75 per cent, foxglove aphid 72 per cent, green peach aphid 60.3 per cent, and lily aphid 48.3 per cent. In another experiment where lots of 20 insects were used the order of efficiency was: lily aphid 80 per cent, green peach aphid 53.3 per cent, celery aphid 40 per cent, and foxglove aphid 36.7 per cent. When single apterous adults were used the order of efficiency was closer to that obtained in the present experiments: lily aphid 8 per cent, celery aphid 1.5 per cent, green peach aphid 0.7 per cent, and foxglove aphid 0.0 per cent. It is evident from the variation in efficiencies shown in these results that any comparative evaluation of vector efficiencies must utilize a standard treatment operating under controlled conditions. Some of the conditions that are desirable and feasible to control are starvation, replication, use of single insects per test plant, watched and timed feeding periods, and uniform virus source plants throughout the replications.

[3] $\bar{x}$ = mean or average.

TABLE 1.—*Results[a] of trials to determine the acquisition threshold period of western celery mosaic virus by 4 species of aphids given different acquisition feeding periods following a starvation period of 30-91 minutes. ($\bar{x}$ — 75.04 min.) and a test feeding period of 1 hour. Thirty replications of 1 plant per test feeding time and 1 insect per plant were used*

Species	Acquisition feeding period in seconds						
	5	10	15	20	25	30	Total
Myzus circumflexus	0	7	24	14	18	12	75
M. solani	0	1	6	9	7	11	34
M. persicae	0	3	4	3	2	2	14
Aphis apii	0	1	4	4	5	2	16
Total	0	12	38	30	32	27	139

[a] Numbers in columns indicate number of plants infected of 30 inoculated.

Results found for the acquisition threshold period agree closely with those obtained for other nonpersistent viruses on which this point has been investigated (5, 14, 17, 18, 19). Differences in vector efficiencies are not parallel to those reported for beet mosaic by Sylvester (19). Although both experiments were similar in their usage of the same 4 species of aphids, preliminary starvation times, acquisition feeding periods, and test feeding periods, there was a marked reversal in the efficiency of transmission between the green peach and lily aphids. In the work on beet mosaic the green peach aphid was the best vector, transmitting with about the same efficiency (36.6 per cent) as the lily aphid showed in this work (41.6 per cent). The lily aphid infected only 3.8 per cent of the plants inoculated with beet mosaic, while the green peach aphid transmitted celery mosaic to about 7.7 per cent of the plants. The other 2 vectors were fairly comparable in their transmission of both viruses.

The hypothesis that differences in vector efficiency are due to the insects acquiring virus at different rates is neither proved nor disproved by these results, nor is the hypothesis that the viruses are inactivated within the insects at different rates. Another equally probable explanation is that the inoculated host plant may influence the chances of obtaining an infection by different species of aphids. Results which were somewhat similar in indicating a compatibility factor between the vector and the plant species inoculated have been reported for transmission of the *Brassica nigra* virus (20).

It was thought that the host plant on which the green peach aphid was reared (*Brassica juncea*) might influence its efficiency in infecting celery, but since Severin and Freitag (11) used celery as a host plant for rearing this aphid and their work with single insects indicated it to be a poor vector, this possibility is questionable. However, the point is of sufficient interest to justify a more critical appraisal.

SUMMARY AND CONCLUSIONS

Experiments were undertaken to determine the ac-

quisition threshold period of western celery mosaic virus for the green peach aphid, *Myzus persicae* (Sulz.); the foxglove aphid, *M. solani* (Kalt.); the crescent marked lily aphid, *M. circumflexus* (Buckt.); and the celery aphid, *Aphis apii* Theob. None of the species was able to acquire the virus in a 5–second feeding period, but all were able to pick up virus in a 10-second period. The lily aphid was the most efficient vector and the green peach and the celery aphids were the least. The 15-second acquisition feeding period was optimum for the lily aphid, the 20-second period for the foxglove aphid; results were inconclusive as to an optimum period for the other species tested.

Adoption of the terms "acquisition threshold period," "acquisition feeding period," and "test feeding period" was proposed since they are largely self-explanatory and are used by a number of workers in the field.

Differences in vector efficiency in transmission of different viruses by the same species were pointed out. It was again hypothecated that a vector-host plant compatibility factor may exist which accounts for these differences in vector efficiency.

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LITERATURE CITED

1. ANDERSON, C. W. 1951. The insect vector relationships of the filaree red-leaf virus, with special reference to a latent period difference between nymphs and adults in Macrosiphum geranicola (Lambers). Phytopathology. **41**: 699-708.
2. CADMAN, C. H. 1951. Studies in Rubus virus diseases. I. A latent virus of Norfolk Giant raspberry. Ann. Appl. Biol. **38**: 801-811.
3. CHAUDHURI, R. P. 1950. Studies on two aphid-transmitted viruses of leguminous crops. Ann. Appl. Biol. **37**: 342-354.
4. FRAZIER, N. W. 1951. New aphid vectors of strawberry viruses. Jour. Econ. Ent. **44**: 258.
5. JENSEN, D. D. 1950. Nasturtium mosaic, a virus disease of Tropaeolum majus L. in California. (Abs.) Phytopathology **40**: 967.
6. KASSANIS, B. 1947. Studies on dandelion yellow mosaic and other viruses of lettuce. Ann. Appl. Biol. **34**: 412-421.
7. MARAMOROSCH, K. 1952. Direct evidence for the multiplication of aster-yellows virus in its insect vector. Phytopathology **42**: 59-64.
8. MELLOR, F. C. AND R. E. FITZPATRICK. 1951. Studies of various diseases of strawberries in British Columbia. II. The separation of the component viruses of yellows. Can. Jour. Bot. **29**: 411-420.
9. PRENTICE, I. W. 1949. Resolution of strawberry virus complexes. III. The isolation and some properties of Virus 3. Ann. Appl. Biol. **36**: 18-25.
10. PRENTICE, I. W. AND T. M. WOOLLCOMBE. 1951. Resolution of strawberry virus complexes. IV. The latent period of virus 3 in the vector. Ann. Appl. Biol. **38**: 389-394.
11. SEVERIN, H. H. P. AND J. H. FREITAG. 1938. Western celery mosaic. Hilgardia **11**: 493-558.
12. SMITH, K. M. AND D. E. LEA. 1946. Transmission of plant viruses by aphides. Parasitology **37**: 25-37.
13. STUBBS, L. L. 1948. A new virus disease of carrots: Its transmission, host range, and control. Aust. Jour. Sci. Res. Series B. Biol. Sci. **1**: 303-332.
14. SWENSON, K. G. 1951. Insect transmission of strains of alfalfa mosaic virus. (Dissertation presented at Univ. of Calif., Berkeley, Calif.)
15. SWENSON, K. G. 1952. Aphid transmission of a strain of alfalfa mosaic virus. Phytopathology **42**: 261-262.
16. SYLVESTER, E. S. 1949. Transmission of sugar beet yellow-net virus by the green peach aphid. Phytopathology **39**: 117-132.
17. SYLVESTER, E. S. 1949. Beet mosaic virus—green peach aphid relationships. Phytopathology **39**: 417-424.
18. SYLVESTER, E. S. 1950. Transmission of Brassica nigra virus by the green peach aphid. Phytopathology **40**: 743-745.
19. SYLVESTER, E. S. 1952. Comparative transmission of beet-mosaic by four aphid species. Phytopathology **42**: 252-254.
20. SYLVESTER, E. S. AND J. N. SIMONS. 1951. Relation of plant species inoculated to efficiency of aphids in the transmission of Brassica nigra virus. Phytopathology **41**: 908-910.
21. WATSON, MARION A. 1938. Further studies on the relationship between Hyoscyamus virus 3 and the aphis Myzus persicae (Sulzer) with special reference to the effects of fasting. Proc. Roy. Soc. Lond. B. **838**: 144-170.
22. WATSON, MARION AND FLORENCE M. ROBERTS. 1939. A comparative study of the transmission of Hyoscyamus virus 3, and potato virus Y and cucumber virus 1 by the vectors Myzus persicae (Sulz), M. circumflexus (Buckton) and Macrosiphum gei (Koch). Proc. Roy. Soc. Lond. B. **127**: 543-576.

TANNIN AS A FACTOR IN THE RESISTANCE OF CHESTNUT, CASTANEA SPP., TO THE CHESTNUT BLIGHT FUNGUS, ENDOTHIA PARASITICA (Murr) A. and A.[1]

Hans Nienstaedt

Young chestnut trees usually escape infection by the chestnut blight fungus, *Endothia parasitica* (Murr) A. and A., until 8 to 12 years. Artificial inoculation is not a dependable means of testing resistance; trees may resist repeated inoculations, only to be attacked naturally at some later date when they show little or no resistance. This has been a serious obstacle in attempts to breed resistant chestnuts. It would be advantageous if a test could be designed to determine the relative resistance of hybrids at an early age. To do so it is necessary to understand the factors involved in blight resistance.

Bramble (4) studied the histology of the bark of infected native chestnut, *Castanea dentata* Borkh. and suggested that the formation of cork layers might be a factor in resistance. Since the fungus penetrated these layers in some cases, he added that in order to be stopped by the cork tissue the fungus must first be weakened by some compound present in the host cells.

Phenolic compounds and the related tannins have often been shown to be factors in disease resistance. Offord (21) correlated the type of tannin in different species of Ribes with their relative resistance to *Cronartium ribicola* Fischer. Onion smudge resistance is caused by the presence of catechol and protecatechuic acid (16, 17), and Rennerfelt (24, 25) proved the fungicidal qualities of phenolic compounds isolated from the wood of different conifers.

The bark of the chestnut contains a high percentage of tannin; consequently a relationship between tannin content and resistance to Endothia canker has been suggested. Cook and Wilson (6) found that certain tannin-containing extracts of the bark of the native chestnut retarded growth of the fungus, while Graves (11) considered the very high tannin content of the roots as the cause of resistance of these parts of the plant. This study is an attempt to elucidate the problem further, by assaying extracts and purified tannins of the bark of 3 species of chestnut with different relative resistance to *Endothia parasitica*.

MATERIALS AND METHODS.—*Isolates of the Fungus and Preparation of the Bark.* Three isolates of the fungus were used. They were all made from cankered American chestnut sprouts growing within a short distance of each other. All isolates reacted similarly to the assays and presumably were from the same strain of the fungus.

For the extractions, bark was collected from 3 species of chestnut, the highly resistant Chinese chestnut, *Castanea mollissima* Bl., the susceptible American chestnut, *Castanea dentata*, and the Japanese chestnut, *Castenea crenata* Sieb. & Zucc., which shows intermediate resistance.

Bark of Chinese and Japanese chestnuts was taken from 1 tree of each species; the trees were attacked by the fungus but showed the resistance typical of these species. Four American trees were used; they were all older suppressed trees and showed no signs of Endothia; however, as similar surrounding trees showed no resistance it seems safe to assume that the trees simply had escaped disease.

The growth habit of the fungus in the Chinese chestnut indicates that a substance responsible for resistance may be concentrated in the inner living bark; consequently the outer dark colored bark and the light colored living bark were kept separate as they were stripped off the lower stem. From the upper stem and the larger branches the bark was removed in its entirety. After drying at room temperature the bark was ground in a Wiley mill with a 15-mesh screen.

Extraction of the bark.—The extraction with the different solvents was performed as follows:

Distilled water: Thirty-five grams (oven dry basis) bark was weighed out and 210 cc. distilled water added. After heating for 2 hours on a hot water bath the liquor, while still hot, was filtered through a Büchner funnel with 2 layers of No. 1 filter paper.

Alcohol (95 per cent): Thirty-five grams (oven dry basis) bark was soaked for 2 hours at room temperature in 210 cc. 95 per cent alcohol and filtered; the filtrate was poured through the bark twice while it remained on the filter. The alcohol was evaporated under reduced pressure at 40–44° C. The residue was then allowed to dry overnight at reduced pressure. Only part of this residue was soluble in water; the remainder was dissolved in 50cc. absolute alcohol. The water fraction was made up to the original volume and used directly for the preparation of the medium. Of the alcoholic extract 23.8 cc. was added to 100 cc. of water and the mixture was concentrated on a steam bath to the original volume of water. The plates were prepared with this solution.

Ether: The original extract was prepared in a manner similar to that described for alcohol. The solvent was evaporated on a steam bath at 45° C. The residue was dried overnight in a desiccator and again dissolved in 10 cc. ether. The plates were prepared as follows: 10 cc. aliquots of potato-dextrose agar solution were sterilized in the autoclave and while still hot 1 cc. of the ether extract was added; the test tube was shaken vigorously and the plates poured.

Purification of the tannins.—The technique used was developed by Kurmeir (15) and recommended by Freudenberg (8) for chestnut and oak.

One thousand grams of the entire bark was extracted

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by alternate soaking in distilled water at 60° C. and pressing in a hydraulic press until the bark gave a very weak reaction for tannins with iron alum.

Subsequent purification of the extracts was the same as that described by Kurmeir and Freudenberg except for the final extraction with ethyl acetate; only this step will be described. The solution was concentrated and extracted for a total of 72 hours with deacidified ethyl acetate. The extraction was carried out in a continuous flow apparatus similar to the one shown by Freudenberg. However, as the water was not cold enough to prevent ethyl acetate from escaping under vacuum the temperature of the ethyl acetate was raised to 80° C., at which temperature the apparatus worked well without the vacuum.

Except for some differences in the particle size of the precipitate obtained with pyridin, the extracts of the 3 different species reacted quite similarly to the purification schedule and the resulting purified tannins were alike in appearance.

Preparation of the media and inoculations.—The standard potato-dextrose agar technique was used for the assays, the toxicity being expressed as a rate of hyphal growth on an agar medium in which the various extracts or tannins had been incorporated.

Inoculations were made from cultures of the fungus growing on a potato-dextrose agar medium. Whenever possible the inoculum for a complete experiment was taken from a single culture. Sometimes, however, it was necessary to use 2 or 3, in which case they were always transfers from the same original culture.

Standard amounts of inoculum were obtained by using a 5 mm. corkborer for cutting the disks. The plates were stacked in the transfer room at 21–23° C. Although not arranged in any special design the plates were shifted around after each measurement in order to avoid border effects.

Collection of data.—The cultures were measured at 24-hour intervals. The mean of 2 perpendicular diameters recorded to the nearest millimeter was used for the computations. In the tables, growth increment is expressed by the regression coefficients (r) which were computed by the method of least squares.

RESULTS.—*Preliminary Experiments.*—Results of one of the assays of the water extracts are in Table 1.

TABLE 1.—*Average increment (regression coefficient r in mm) in growth of Endothia parasitica in water extracts of different parts of the bark of Chinese, Japanese and American Chestnut*

Species	Bark Type Inner	Outer	Entire
Chinese	3.22	6.61	6.45
Japanese	7.06	5.98	6.86
American	9.34	6.61	8.06
Control		8.93	

Correlation between the toxicity of the extracts and the actual resistance of the 3 species can be seen in the results of the assays of the inner bark extracts. Tested statistically the differences between the 3 species were highly significant; 2 similar experiments gave comparable results. The experiments indicate that Chinese and Japanese chestnut barks contain materials extractable in water and possessing antibiotic properties. Furthermore, it seems probable that the toxic compound is concentrated in the living portion of the bark.

It will be shown later that the type of tannins present in the Chinese chestnut is different from that in the Japanese and American species. This difference is reflected in the data of Table 1. While in the Chinese bark the toxicity increases from the outer dead bark to the inner bark the situation is reversed in the Japanese and American bark extracts. Apparently changes take place in the chemical constituents of the bark when it dies depending on the original compounds present. In the Chinese chestnut the result is a decrease in toxicity while in the 2 other species it is an increase.

Field observations do not indicate that the outer bark is more resistant to invasion from the fungus than the inner bark in the Japanese and American chestnut; therefore, it seems that the toxic agents are inert under natural conditions, but are released during the extractions.

Results of the assays of the alcohol extracts of the 3 species are in Table 2.

TABLE 2.—*The growth (regression coefficient r in mm) of E. parasitica in 2 fractions of alcohol extracts of the bark of chestnuts*

Species	Water soluble frac. of alc. ext.	Alcohol soluble frac. of alc. ext.
Chinese	5.78	6.15
Japanese	6.18	5.12
American	6.82	5.24
Controls	9.73	9.55

Both fractions of the alcohol extracts retarded growth of the fungus. Toxicity of the water-soluble fractions fall in the same order as resistance in the 3 species, but differences in toxicity were relatively not as great as for the water extracts. Tested statistically by the t-test the difference between any 2 of the extracts was significant at the 1 per cent level. The toxicity of the alcohol-soluble fraction did not follow this pattern.

The ether extract from all species had an antibiotic effect but that from the Japanese species was much more toxic ($r = .34$ mm) than that from the Chinese ($r = 1.49$ mm). Thus, toxicity again did not follow the pattern of resistance. Therefore this extract probably contains no factors which play a role in resistance to the disease.

The water extract will predominately contain glucosides, including tannins, starch, pectins and sugars. Of these it seems most likely that the toxic agent will be found among the tannins, other glucosides or re-

lated compounds, but other compounds might be present which could account in part for the toxicity of the extracts.

Assay of purified tannins.—Purified tannins were assayed in 4 concentrations starting with 1.20 per cent and diluting 3 times to get each subsequent concentration; the approximate concentrations in percentages were 1.20, 0.40, 0.13 and 0.04. The results are in Table 3.

TABLE 3.—*The growth (regression coefficient r in mm) of E. parasitica in different concentrations of purified tannins derived from the bark of Chinese, Japanese and American chestnut*

Conc. per cent	Species		
	Chinese	Japanese	American
1.20	0.0	8.55	8.79
0.40	--	9.99	10.29
0.13	10.05	10.70	10.40
0.04	10.19	10.67	10.93
Control		10.24	

The cultures were measured over a period of 7 days. During this interval no measurable growth developed in the 1.20 per cent Chinese tannin. After 11 days these cultures were measured again and showed an average diameter of only 10.33 mm. The growth of the fungus on the 0.40 per cent Chinese tannin was quite abnormal; instead of spreading out radially from the inoculum long, narrow arms spread very rapidly from the center, and from them the intervening spaces were gradually filled (Figure 1). The measurements from this series have been left out as they were abnormal. Transfers were made from both these series to standard potato-dextrose agar on the eleventh day; the resulting cultures were normal in all respects. The tannins from the Japanese and American chestnut retarded the growth of the fungus only in the 1.20 per cent media, and no difference could be demonstrated between them.

Concentration of tannin in the crude water-extracts.—In order to compare the results of the assays of the crude water extracts and the purified tannins, new extracts were made. They were assayed with the fungus, and their tannin concentration was found using the standard hide-powder method (2). This is an empirical method which does not distinguish between different types of tannins and includes many non-tannin compounds in the estimate. Results are in Table 4.

The correlation between the tannin concentration and the growth of the fungus seems to be well established, especially so when considering the earlier experiments in which the tannin content undoubtedly was comparable to the values in Table 4. For the outer bark the correlation is not apparent. However, the results from the assay with the inner bark are probably the most interesting, because this seems to be the part of the bark which prevents further advance of the fungus.

Qualitative tests of the extracts.—The crude water extracts of the bark and the purified tannins were subjected to the qualitative formaldehyde-hydrochloric acid test (3, 7). The test was repeated several times and always with comparable results. It is used in distinguishing between pyrogallol and catechol tannins. By boiling tannin solutions with a mixture of formaldehyde and concentrated hydrochloric acid under a reflux condenser, catechol tannins are precipitated while pyrogallol tannins are only partially precipitated, if at all. After cooling, sodium acetate is added to the filtrate; if a blue color develops with iron alum, pyrogallol tannin is present. With mixtures both a precipitate and a blue color is formed.

The crude extract of the Chinese chestnut gave a small brown precipitate after boiling while none was formed in the purified tannins; the filtrates from both gave a purple color with iron alum. The crude extracts of both Japanese and American chestnut gave an immediate precipitate when HCl was added; the precipitate turned red during boiling. The purified tannins gave a precipitate after boiling; both filtrates gave purple colors with iron.

Results show that the tannins in Japanese and American chestnut are mixtures of pyrogallol and catechol tannins. Chinese chestnut tannins apparently either are mixtures containing only small amounts of catechol tannin, or they are pure pyrogallol tannin a little of which is precipitated during the boiling.

Bark samples of an additional 63 trees of Chinese, Japanese, American, and hybrid origin were also tested qualitatively. It was first ascertained that no differ-

TABLE 4.—*The growth (regression coefficient r in mm) of E. parasitica on chestnut bark extracts with known tannin concentration determined by the hide-powder method*

Species	Inner Bark				Outer Bark			
	Test 1[a]		Test 2[a]		Test 1[a]		Test 2[a]	
	Tannin %	r mm	Tannin %	r mm	Tannin %	r mm	Tannin %	r mm
Chinese	1.32	1.55	1.30	10.49	1.39	5.18	1.51	8.29
Japanese	1.01	3.06	1.07	10.36	0.52	4.72	0.59	8.23
American	0.60	6.89	0.74	11.23	0.55	8.62	0.60	9.39
Control		11.19		11.23		11.19		11.23

[a] A transfer 31 days old was used in Test 1 and one 15 days old in Test 2. From other experiments it has been observed that the older the inoculation, the more sensitive it is to the extracts.

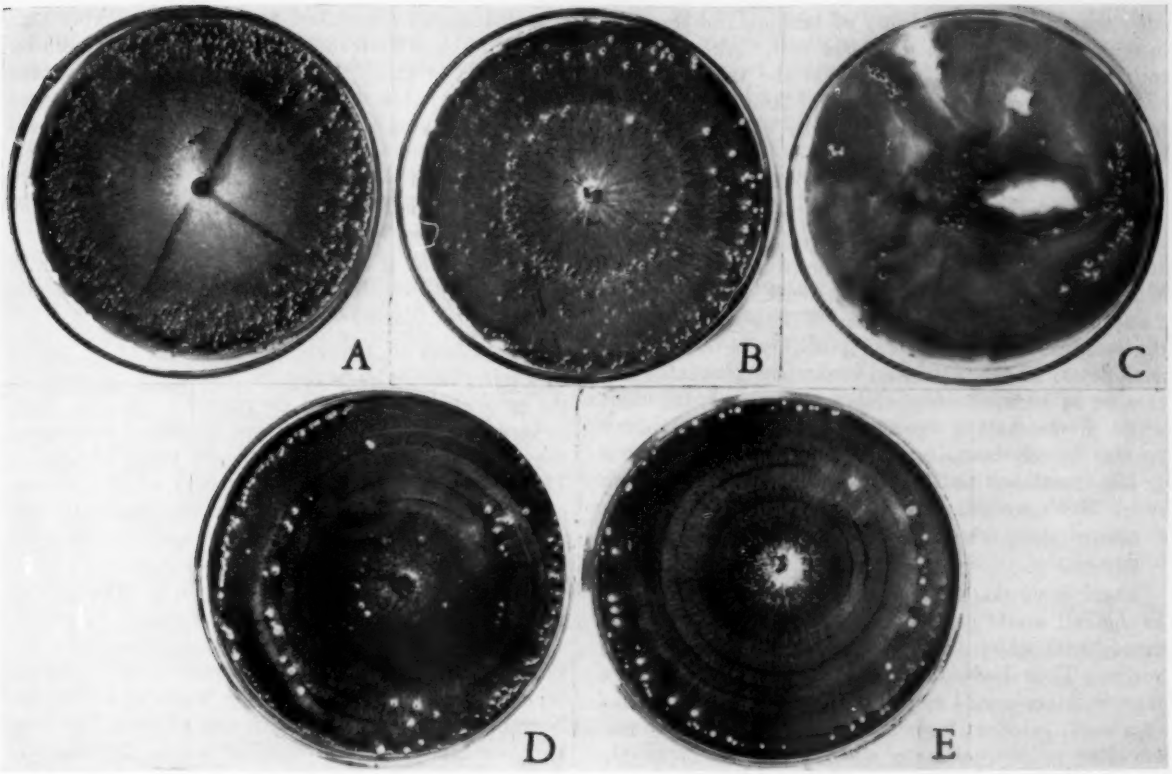

FIG. 1. Representative cultures of *E. parasitica* growing on the purified tannins from the bark of American, Japanese and Chinese chestnut: 0.4 per cent tannin of American chestnut A), Japanese chestnut B) and Chinese chestnut C); 1.2 per cent tannin of American chestnut D) and Japanese chestnut E). The white spots with dark centers are the spore pustules. Note the abnormal growth on the medium containing 0.4 per cent tannin from Chinese chestnut.

ences occurred in the type of the tannins in the bark at different points in the tree. Thereafter all but 3 samples were taken from 5–10 year old branches in the crown.

Samples from 12 Chinese and 11 American trees all gave reactions similar to those described above for the crude water extracts of these species. Of the 10 Japanese samples 9 reacted as described, but 1 gave a precipitate only after boiling.

Twenty-nine bark samples were taken from hybrids. The trees were 12 years or more of age, and from their reaction to the blight fungus they were classed in 4 groups of resistance: high, medium, low and no resistance. No resistance would be similar to the American chestnut and high would be as resistant as the best Chinese trees.

The result of the analyses indicated no simple correlation between type of tannin and resistance. Thirteen Chinese × (Japanese × American) hybrids, 6 with high, 6 with medium and 1 with low resistance, were tested; all gave the typical Chinese reaction. Four Chinese × American hybrids reacted similarly except that 1 sample from a non-resistant tree formed a red precipitate after boiling rather than a brown. Of 5 Japanese × American hybrids tested, 2 reacted like the parents giving an immediate precipitate. The other 3 samples were taken from 2-year-old sprouts; they gave a precipitate after boiling, as in the Chinese bark samples. Apparently an unknown variant was encountered by collecting the samples from the young sprouts. Five backcrosses or intercrosses of Japanese × American hybrids were tested; they all reacted in the same way as the parents.

DISCUSSION.—Results show that the tannin content of the bark is a factor in the resistance of the chestnut species. It is not, however, a simple correlation between increasing concentration and increasing resistance; this would fail to account for the results of the assay with the purified tannins. Furthermore, the total concentration of tannin in the bark of the 3 species is very similar; 13.3–17.8 per cent in the Chinese (5), 10.9–20.1 per cent in the Japanese (Diller, unpublished data), and 10.3–13.6 in the American species (9). These concentrations overlap and if the correlation between tannin concentration and resistance was a simple one the same overlapping in resistance should occur. Field examinations show no overlapping. It therefore becomes necessary to consider other factors in order to explain resistance completely.

Nierenstein (20) has summarized our present knowl-

edge regarding the solubility of tannins and the way in which they occur in the plant cell. Although not conclusive the indications are that the tannins occur either in a free condition in special tannin-vacuoles or form addition compounds with other colloids. In the latter case their solubility will depend on how closely the colloids are bound together.

The concentration of the tannins in crude water extracts (Table 4) from Chinese chestnut is more than 100 per cent greater than in the American extract. When compared with the total concentration in the 2 species this seems to indicate a different solubility of the tannins. The values can probably be interpreted as follows: in the American chestnut the tannins are present as addition compounds bound closely together, while in the Asiatic species as free or as combined tannins loosely bound and therefore more soluble. It is also possible that the tannins are present in both types of chestnut partly in a free and partly in a combined stage, the proportion of free tannins being higher in the Asiatic chestnuts.

There is no doubt that the condition of the tannins in the cell would have a pronounced effect on the efficiency with which they would check the parasitic invader. Thus Huber (14) found that while a pure tannin solution will retard fermentation when added to a sugar solution, fermentation proceeds at a normal, or close to normal, rate when a gum-arabic-tannin complex is added. Cook & Wilson (6) found that *E. parasitica* "gave good growth of mycelium and scant spore formation when grown on an agar medium containing 0.6 per cent tannin, but a less toleration to tannin when grown on a liquid medium."

Discussing the work of Huber, and Cook and Wilson, Nierenstein (20) concludes: "If the tannin in a host plant is present in the form of a compound with other colloids, it seems likely that it will have less inhibiting effect on parasitic fungi."

A comparison of the concentration of tannins in the crude extract and purified tannin mediums and the corresponding growth of the fungus shows that the effect of the purified tannin of Chinese chestnut has about the same magnitude as the effect of the crude extract. For the American extracts, on the other hand, a 0.60 per cent tannin concentration in the crude extract retarded the growth of the fungus considerably, while as much as 1.2 per cent pure tannin retarded the growth only slightly. The reason for this is not clear. The hide powder method is a rather empirical technique which will include in the estimate of tannin related compounds, coloring matter, and the like. Some of these impurities, estimated as tannin, may well possess antibiotic effects; after their removal a higher concentration of the pure tannin would be required to bring about a toxic effect.

Possible correlation between the qualitative differences in the purified tannins and their relative toxicity. —Information regarding the nature of the tannins of the chestnut is limited. The tannin in the bark of European chestnut, which especially has been studied, is a mixture of pyrogallol and catechol tannins (22, 23). In the American chestnut a similar condition is found (9, 12). The only reference to Chinese chestnut (13) describes the tannins of the leaves and bark as belonging to the pyrogallol group. The quantitative tests seem to substantiate this classification although a small amount of catechol tannins probably is mixed in. No descriptions of the tannins in the Japanese species have been found.

The reactions of the Japanese and American tannins to the formaldehyde-hydrochloric acid test indicates that they are mixtures.

The toxicity of tannins is considered to be caused by their phenolic constituents which are released by the enzymatic activities of the parasite (19, 21). There is very little information in the literature concerning the relative toxicity of the different types of tannins. The complexity and varied chemistry of the tannins, combined with the complexity of the enzymatic systems of the fungi, leaves plenty of scope for differential toxicity of a certain type of tannin against different fungi or for variations in the behavior of different tannins against the same fungus. Tannase is an enzyme complex of varied constitution. The tannase of a certain fungus may be able to decompose only a certain type of tannin as shown by the work of Fuller and Nierenstein (10). They found that whereas *Polyporus spp.* decomposed only pyrogallol tannin, *Collybia spp.* decomposed the catechol tannin, and only this type of tannin could be found in its hyphae.

Freudenberg and Walpuski (8) studied the decomposition of tannins of European chestnut by Aspergillus-tannase and obtained traces of gallic acid, ellagic acid and sugars. No information is available concerning the effect of tannase from other fungi on chestnut tannin; neither do we know what the tannase decomposition of the tannins of American, Japanese and Chinese chestnut may yield, or the type of enzymes produced by Endothia. Until additional information is available the following is suggested as a working hypothesis explaining the role of the tannin quality in resistance to Endothia.

The tannase complex of *E. parasitica* decomposes pyrogallol tannins and in doing so produces a simple phenol which is toxic to the fungus. The tannins in the bark of Chinese chestnut belong to the pyrogallol group, whereas the barks of American and Japanese chestnut contain a tannin which is a mixture of pyrogallol and catechol tannins. In media where the overall concentration of tannin is the same, the concentration of the active ingredient may be considerably lower in the media containing the extract from the bark of Japanese and American chestnut and their toxicity therefore similarly reduced.

It is possible that the phenol which is the actual cause of toxicity is pyrogallol. This is probably a breakdown product of pyrogallol tannin, and the compound is extremely toxic to the fungus. An assay of pyrogallol, catechol, phloroglucinol, gallic acid, protecatechuic acid, and digallic acid showed that pyro-

gallol retarded the growth of Endothia considerably in a 1:6,400 and a 1:25,400 dilution. Catechol retarded growth at 1:1,600, but the 2 weaker concentrations were practically without effects.

The solubility and type of tannins are the 2 factors which determine the resistance of a tree. American chestnut has tannins of low solubility and they are mixtures; this species is therefore least resistant. In the Japanese species the tannins are also mixtures but they are more soluble than in the native trees and this species is more resistant. The highest degree of resistance is found in the Chinese chestnut where the tannins belong to the more toxic pyrogallol type and also possess a high degree of solubility.

In the hybrids the situation is further complicated. It seems that the 2 factors, type and solubility of the tannins, are interacting and masking each other. However, much additional information is needed to clarify this.

Still more research will be needed to get a complete picture of disease resistance in chestnut; thus, other compounds may play a role in the chemical resistance. Also, the wound tissue formation must be studied in order that the earlier suggestions by Bramble (4) be definitely proved or disproved.

SUMMARY

The role of tannins in disease resistance of chestnut *Castanea spp.* attacked by *Endothia parasitica*, the chestnut blight fungus was studied. Pulverized bark of *Castanea dentata*, the American chestnut, *Castanea crenata*, the Japanese chestnut, and *Castanea mollissima*, the Chinese chestnut, was extracted with water, alcohol and ether. Assays of the extracts with the fungus indicated that the bark of the more resistant species, *C. mollissima* and *C. crenata*, contains antibiotic substances in high enough concentrations to cause a retardation of the growth of the fungus.

The tannins of the bark of the 3 species were purified. In assays they all retarded the growth of the fungus when present above a certain concentration. There was no difference between the effects of the tannin from Japanese and American chestnut, but that from the Chinese chestnut was much more toxic.

The total concentration of the tannins in the bark of the 3 species is not correlated with the relative resistance of the species. In the water extracts, however, the concentration of tannins was larger in extracts from the resistant species. This may indicate a differential solubility of the tannins in the 3 species.

A qualitative test seems to indicate that the tannin in Chinese chestnut is a pyrogallol tannin, whereas the other 2 species contain a mixture of catechol and pyrogallol tannin.

It is suggested that the relative resistance of the 3 species, at least in part, is the result of the differential solubility and qualitative differences among the tannins in the 3 species studied.

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LITERATURE CITED

1. ANDERSON, P. J., AND H. W. ANDERSON. 1913. The chestnut blight fungus and a related saprophyte. Pa. Chestnut Tree Blight Commission, Bul. 4.
2. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1945. Official and tentative Methods of Analysis. Ed. 6. 932 pp.
3. ATKIN, W. R., AND F. G. THOMPSON. 1937. Procter's Leather Chemists' Pocket-book. A short compendium of analytical methods. Ed. 3. New York and London. 394 pp.
4. BRAMBLE, W. C. 1936. Reaction of chestnut bark to invasion by Endothia parasitica. Am. Jour. Bot. **23**: 89-94.
5. CLARKE, I. D., AND R. W. FREY. 1932. Tannin data on hairy Chinese chestnut trees grown in the United States. Jour. Am. Leather Chemists' Assoc. **27**: 206-215.
6. COOK, M. T., AND G. W. WILSON. 1916. The influence of the tannin content of the host plant on Endothia parasitica and related species. New Jersey Agr. Exp. Sta. Bul. 291.
7. FREUDENBERG, K. 1932. Die natürlichen Gerbstoffe. In G. KLEIN. Handbuch der Pflanzenanalyse II. 344-407.
8. FREUDENBERG, K. AND H. WALPUSKI. 1921. Der Gerbstoff der Edelkastanie (8. Mitteilung über Gerbstoffe und ähnliche Verbindungen) Berichte der Deutschen Chemischen Gesellschaft. 54B: 1695-1700.
9. FREY, R. W., AND L. R. LEINBACK. 1925. The distribution of tannin in the American Chestnut tree with particular reference to stumps and roots. Jour. Am. Leather Chemists' Assoc. **20**: 457-470.
10. FULLER, B. S., AND M. NIERENSTEIN. 1944. A macro and micro method for the estimation of pyrogallol tannins and catechol tannins in presence of each other. The Analyst **69**: 374-375.
11. GRAVES, A. H., 1926. The cause of the persistent development of basal shoots from blighted chestnut trees. Phytopathology **16**: 615-621.
12. GREAVES, T. G. 1918. Color test of chestnut bark. (Abs.) Jour. Am. Leather Chemists' Assoc. **13**: 467-469.
13. HSU, S. H., H. L. CHANG, AND Y. W. TIEN. 1940. A survey of common materials containing vegetable tannins found in Szechwan. (Abs.) Jour. Chem. Eng. China **7**: 26-28.
14. HUBER, H. 1929. Über den Zustand und die Rolle der Gerbstoffe in der Pflanze. Jahrbücher für Wissenschaftliche Botanik **70**: 278-327.
15. KURMEIR, A. 1927. The tannin of the native oak and the chestnut. Collegium: 273-288.
16. LINK, K. P., A. D. DICKSON AND J. C. WALKER. 1929. Further observations on the occurrence of protecatechuic acid in pigmented onion scales and its relation to disease resistance in the onion. Jour. Biol. Chem. **84**: 719-725.
17. LINK, K. P., AND J. C. WALKER. 1933. The isolation of catechol from pigmented onion scales and its significance in relation to disease resistance in onions. Jour. Biol. Chem. **100**: 379-383.
18. NEWTON, R., J. V. LEHMAN, AND A. E. CLARK. 1929. Studies on the nature of rust resistance in wheat. Can. Jour. Res. **1**: 5-35.
19. NEWTON, R., AND J. A. ANDERSON. 1929. Studies on

the nature of rust resistance in wheat IV: Phenolic compounds of the wheat plant. Can. Jour. Res. **1**: 86-99.
20. NIERENSTEIN, M. 1934. The natural organic tannins. History, Chemistry Distribution. London. 319 pp.
21. OFFORD, M. R. 1940. The function of tannin in host parasite relationships with special reference to Ribes and Cronartium ribicola. U.S. Dept. Agr. Bur. Ent. and Pl. Quar. E-518.
22. PONTE, A. 1932. Differential characteristics of chestnut tannin extracts obtained from the bark and from the debarked wood. (Abs.) Boll. uff. Staz. sper. ind. pelli. mat. concianti **10**: 42-59.
23. PONTE, A. 1932. Comparison of chestnut bark and chestnut wood extract. (Abs.) J. Intern. Soc. Leather Trades Chem. **16**: 394-403.
24. RENNERFELT, E. 1945. The influence of the phenolic compounds in the heartwood of scotch pine (Pinus silvestris L.) on the growth of some decay fungi in nutrient solution. Svensk Botanisk Tidskrift **39**: 311-318.
25. RENNERFELT, E. 1948. Investigations of thujaplicin, a fungicidal substance in the heartwood of Thuia plicata. D. Don. Physiologia Plantarum **1**: 245-254.

ALFALFA YELLOW MOSAIC VIRUS SYSTEMICALLY INFECTIOUS TO BEANS [1]

W. J. Zaumeyer

Among a collection of virus-infected alfalfa plants made in Idaho in 1950, there was one with a bright yellow leaf mottling collected near Jerome. Expressed juice from this plant produced both necrotic local and systemic symptoms when inoculated on beans (12). Experimental studies showed that, in addition to an alfalfa virus, a strain of cucumber mosaic virus was involved. The cucumber mosaic virus was separated from the complex by passage through cucumber, which was resistant to the alfalfa virus. Studies on the cucumber mosaic virus isolate will be discussed in another paper. The alfalfa virus was separated from the complex by making single necrotic lesion isolations from beans. This was done by piercing single local leaf lesions with a sterile needle and then puncturing healthy Pinto bean leaves with this needle. Expressed juice from infected leaves thus inoculated produced necrotic local lesions on rubbed leaves of Pinto plants (Fig. 1, C) a few days after inoculation and, later, mottling and necrosis of the trifoliate leaves (Fig. 1, G and H). Failure of the expressed juice to infect cucumber plants showed that the isolate had been freed of the cucumber virus component. This paper compares this new strain of alfalfa mosaic virus with its other strains. The disease in alfalfa is named alfalfa yellow mosaic and the virus is classified as *Marmor medicaginis* var. *flavovarians* n. var.

METHODS AND MATERIALS.—The experimental work was conducted in the greenhouse at approximately 20 to 27° C. The viruses[2] were maintained on Pinto U. I. No. 78 bean and Turkish tobacco plants. All plants were inoculated in the early stages of growth and carborundum powder No. 320 was used as an abrasive in the transfers.

SYMPTOMS AND HOST RANGE.—In order to compare readily host reactions to and symptomatology of alfalfa yellow mosaic virus with those of the more recently described strains of alfalfa mosaic, such as yellow patch virus of Ladino clover (5), tuber-necrosis virus of potato (7), and yellow dot virus of bean (9), many of the species tested with these viruses by the several investigators were also inoculated with the alfalfa yellow mosaic virus.

Alfalfa, *Medicago sativa* L. The symptoms produced on this host are similar to those of typical alfalfa mosaic. Both viruses produce chlorotic interveinal streaking of leaves, but the yellowing produced by alfalfa yellow mosaic virus is more intense. The mottling caused by typical alfalfa mosaic virus is masked at high temperatures, but this was not the case with yellow mosaic virus in the field. It was not possible to infect alfalfa artificially with alfalfa yellow mosaic virus in the greenhouse.

Bean, *Phaseolus vulgaris L.* On Bountiful bean, small, yellow chlorotic spots (Fig. 1, E) appeared on the inoculated leaves 4-5 days after inoculation and 10 days later a mottle was noted on these leaves. The other strains of alfalfa mosaic virus, except yellow dot virus, have been reported to produce only necrotic local lesions on this variety. The yellow dot virus produced no lesions on inoculated leaves, no mottling on trifoliate leaves, and only a few yellow spots. The trifoliate leaf mottling caused by alfalfa yellow mosaic virus was intense and resembled the symptoms of yellow bean mosaic (Fig. 1, I). Later, small yellow spots, 1–2 mm. in diameter and resembling the spots produced by yellow dot and yellow stipple viruses on some varieties, appeared (9, 13).

On other bean varieties such as Pinto and Great Northern, the local lesions, produced 2–3 days after inoculation, are mostly 3–4 mm. in diameter and very irregular in outline with stippled necrotic borders (Fig. 1, C and F). When only a few lesions are produced on a leaf they are much larger, some attaining a diameter of 15 mm. They are reddish brown and frequently have light-green centers. Local infection is frequently expressed as a necrosis of the veins rather than definite local spots. Later, the petioles, pulvini, and stems become necrotic. Veinlet necrosis of

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Appreciation is expressed to H. H. Fisher for assistance in certain phases of these investigations.

[2] The yellow patch virus of Ladino clover was supplied by K. W. Kreitlow from locally collected infected clover plants. H. Rex Thomas furnished the culture of the yellow dot virus.

the trifoliate leaves, together with interveinal reddish necrotic spots, frequently develops. Such infected leaves seldom are killed, but are often mottled, malformed, rugose, curled downward, and frequently blotched with large yellow areas (Fig. 1, D). On a few varieties, some of the pods are gnarled and malformed, resembling those infected with pod distorting virus (3). In other cases they develop reddish necrotic rings, similar to those on pods infected by the red node virus (10).

Tobacco, *Nicotiana tabacum* L. On inoculated leaves of the Turkish variety, small white necrotic arcs and rings are usually produced. Sometimes the lesions appear as definite tan circular spots 3–4 mm. in diameter. A mild mottling later develops on the upper leaves but as the plant becomes older the mottling is masked. Infected plants are slightly chlorotic and stunted. Frequently, on older leaves, a white stippling of the oak leaf pattern is observed along the veins (Fig. 1, A). The typical alfalfa mosaic virus, in contrast, produces fairly severe mottle symptoms on young plants and also white necrotic lines of the oak leaf pattern on old infected plants.

Nicotiana glutinosa L. Small gray necrotic local lesions are formed on the inoculated leaves. Upper leaves are mildly mottled and the plant becomes somewhat chlorotic and stunted.

Nicotiana rustica L. Infected plants are moderately mottled.

Datura stramonium L. No local lesions are produced. The mild systemic mottle is of the green vein banding type. On older leaves small, yellow chlorotic spots with brownish centers are formed.

Pepper, *Capsicum frutescens* L. var. California Wonder. No infection.

Tomato, *Lycopersicon esculentum* Mill. var. Marglobe. No infection.

Petunia, *Petunia hybrida* Vilm. No infection.

Hyacinth bean, *Dolichos lablab L.* Small local lesions; very mild systemic mottle and abundant yellow spots on upper leaves.

Soybean, *Glycine max* (L.) Merrill var. Lincoln. Systemic yellow mottle.

White Lupine, *Lupinus albus* L. Small dark local lesions; no systemic infection.

Tepary bean, *Phaseolus acutifolius* Gray var. *latifolius* Freeman. Few very small, dark, necrotic local lesions develop on the inoculated leaves. No systemic infection.

Lima bean, *Phaseolus lunatus* L. var. Henderson Bush. Very mild systemic mottle.

Pea, *Pisum sativum* L. var. Laxton Progress, Early Perfection, Perfected Wales and others. Systemic stem streaking; also small pin point necrotic lesions on inoculated leaves. No mottle.

White Sweetclover, *Melilotus alba* Desr. Mild mottle.

Crimson clover, *Trifolium incarnatum* L. Mild mottle.

Red clover, *Trifolium pratense* L. No infection.

White clover, *Trifolium repens* L. Mild mottle.

Broad bean, *Vicia faba* L. Reddish-brown local lesions on inoculated leaves followed by systemic necrotic spots and rings on upper leaves; such leaves were frequently mottled, twisted, and bronzed. Stem necrosis and killing of terminal growing point often occurs.

Cowpea, *Vigna sinensis* (Torner) Savi var. Black Eye. Abundant, reddish-brown medium-sized primary lesions, followed by intense yellow mottling and malformation of later developing leaves, are produced (Fig. 1, B).

Cucumber, *Cucumis sativus* L. var. White Spine. No infection.

Balsam, *Impatiens holstii* Engler and Warb. Small, dark-colored local lesions; no systemic infection.

Snapdragon, *Antirrhinum majus* L. Moderate-sized grayish ringed local lesions; mild mottle.

Zinnia, *Zinia elegans* Jacq. var. Sunshine Tint. Mild chlorotic mottle.

Reaction of bean varieties.—Because of the severe infection produced by alfalfa yellow mosaic virus on a number of bean varieties, 24 varieties, representative of different types of snap and dry beans, were inoculated.

On Blue Lake 231, Great Northern U. I. 16 and 31, Michelite, Pinto U. I. 78, Red Mexican U. I. 32, Robust and Small White fairly large necrotic local lesions, severe systemic mottle and some necrosis of the trifoliate leaves were produced by the virus. On Blue Lake Regular, Blue Lake 65, Great Northern U. I. 123 (Fig. 1, F), Ideal Market, and Kentucky Wonder White, similar local lesions were noted but the systemic mottling was mild. On Idaho Refugee, Stringless Green Refugee, Tendergreen, Topcrop, and Unrivalled Wax, numerous small, dark necrotic local lesions were produced on the inoculated leaves and a fairly severe mottle and chlorosis on the trifoliate leaves. Bountiful, Landreth Stringless Green Pod, Logan, Low Champion, U. S. 5 Refugee, and Black Valentine showed chlorotic local lesions and mottle of the inoculated leaves. The trifoliate leaves were systemically mottled and chlorotic. Large yellow blotched areas were commonly noted. Bountiful had the most intense mottling. Under certain conditions the chlorotic local lesions became slightly necrotic but not nearly to the same extent as on the varieties that showed only the necrotic lesions on the inoculated leaves.

Properties of the virus.—The physical properties of alfalfa yellow mosaic virus were determined, using freshly expressed juice of infected beans as test material and local lesion production on Pinto bean leaves as a measure of virus activity remaining.

The virus was inactivated between 65 and 70° C. in 10 minutes and withstood a dilution of 4000× but not of 5000×. It was infectious after 72 hours' storage *in vitro* at 18° C. but not after 96 hours'.

Cross Protection.—After plants of the varieties Bountiful, Logan, Low Champion and Unrivalled Wax had become systemically infected with alfalfa yellow mosaic virus they were inoculated with the yellow

TABLE 1.—*Summary of host reactions to reported strains of alfalfa mosaic virus and thermal inactivation points*

Virus	Type of infection	Reaction [a] of— *Phaseolus vulgaris* var. Bountiful	*Nicotiana tabacum*	*Nicotiana glutinosa*	*Datura stramonium*	*Cucumis sativus*	*Vigna sinensis* var. Black Eye	*Capsicum frutescens*	*Pisum sativum*	*Vicia faba*	*Petunia hybrida*	*Trifolium pratense*	Thermal inactivation point (°C.)
Alfalfa mosaic (8, 11) [b]	Local	+ N	+	—	—	—	+	+	—	+	+	—	60-65°
	Systemic	—	+	+	+	+	—	+	+ M	+	+	+	
Potato calico (7)	Local	+ N	—	—	—	—	+	+	—	+		—	53-55°
	Systemic	— D	+	+	+	+	—	—	+ MN	+		+	
Pepper mosaic (1)	Local	+ N	+	+	+	—		+	+ K	+	+	—	60-65°
	Systemic	—	+	+	+	+		—	+ M	+	+	+	
Yellow patch (5)	Local	+ N	—	+		—	+	+	+ K	+	—	—	60-65°
	Systemic	—	+	+		—	—	—	+ M	+	+	+	
Tuber necrosis (7)	Local	+ N	—	—	—	—	+	+	+ N				53-55°
	Systemic	— D	+	+	+	+	—	—	—	+			
Yellow dot (9)	Local	—	—	—	—	+	+	—	—	+		—	62-64°
	Systemic	+	+	+	+	+	+	—	+ M	+		+	
Alfalfa yellow mosaic	Local	+ C	+	+	—	—	+	—	—	+	—	—	65-70°
	Systemic	+ N	+	+	+	—	+	—	+ N	+	—	—	

[a] + = infectious; — = not infectious; C = chlorotic; D = occasionally systemic causing necrotic die back of tip and stem; K = killing of inoculated leaves; M = mottle; N = necrotic.

[b] Figures in parenthesis indicate citation to literature from which data was obtained.

patch virus, which produces local lesions on these varieties. Local lesions of the latter virus failed to develop on the previously infected plants, whereas on the healthy checks they were produced abundantly. After Pinto U. I. 78 had been systemically infected with yellow dot virus, mottled leaves were inoculated with alfalfa yellow mosaic virus. No local lesions were produced on these leaves but many were on the healthy checks. The results suggest that yellow patch, yellow dot, and alfalfa yellow mosaic viruses are related.

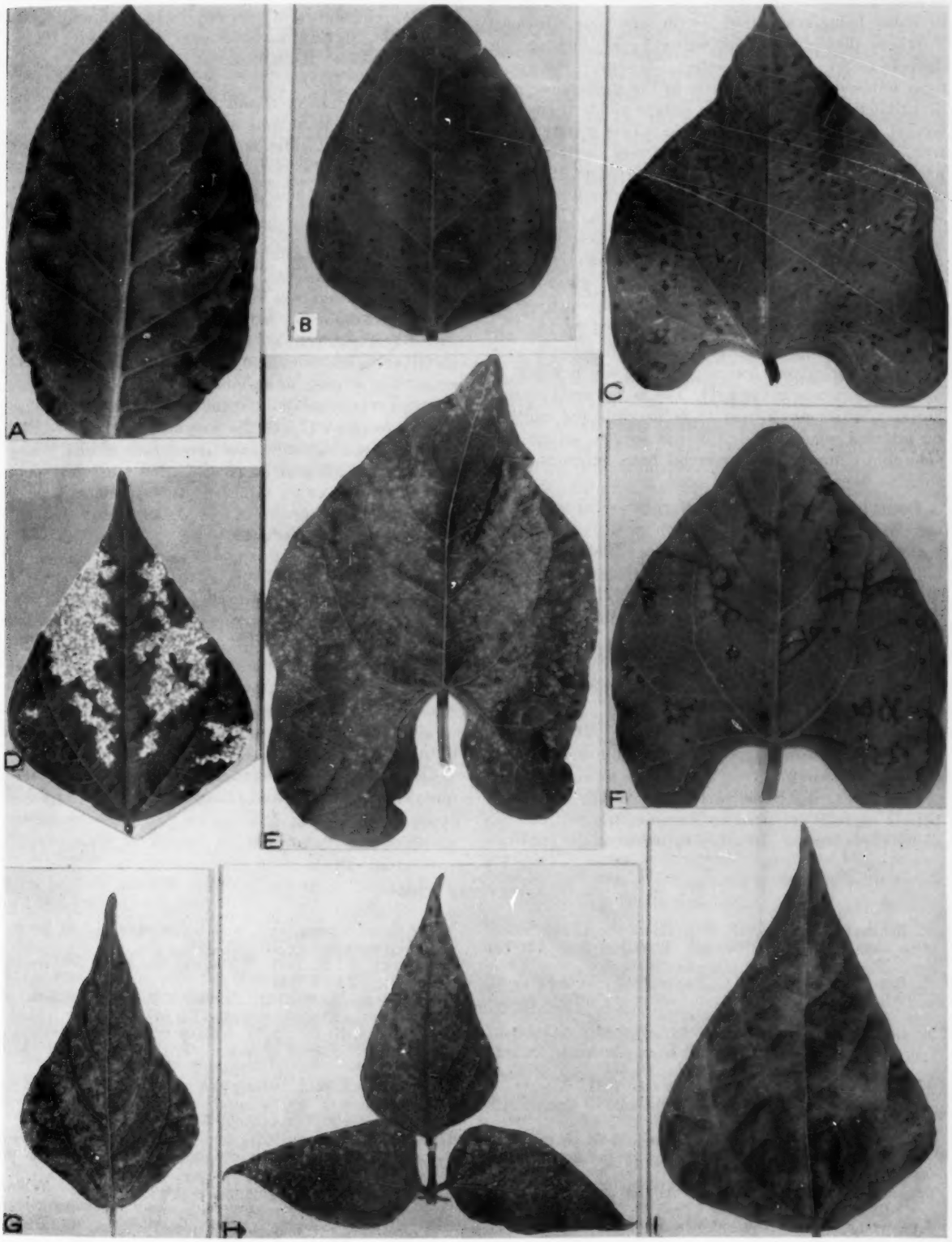
DISCUSSION.—During the past 14 years a number of strains of alfalfa mosaic virus have been described (1, 2, 5, 6, 7, 8, 9, 11). On the basis of symptoms, host range, physical properties and cross protection tests, it is evident that alfalfa yellow mosaic virus is another strain in this group. Table 1 lists the presently described strains of alfalfa mosaic virus, with the exception of alfalfa N virus since very little has been published on its host range and the reactions of certain host plants. The reactions were recorded from publications of the various authors who described the viruses. Although there are many host range similarities between alfalfa yellow mosaic virus and the other strains of alfalfa mosaic virus, there are certain differences. Possibly the most striking is its ability to produce consistently on beans both local necrotic lesions and systemic necrosis and mottling. None of the other viruses in the group produce systemic infection except yellow dot virus which produces more veinal necrosis than local spots and a systemic mottle much milder than that of the new strain.

Alfalfa N, potato calico, and tuber-necrosis viruses also produce both local and systemic infection on bean but the last 2 do so only occasionally. These cause necrosis of the stem and die-back of the growing point (4, 6). In contrast, alfalfa yellow mosaic virus produces intense systemic mottle, necrotic and yellow spots and blotches on the leaves, and necrosis of the stem of many varieties but does not kill the growing point. The thermal inactivation points are approximately 50°-55° C. for alfalfa N virus (6) and 53°-55° C. (7) for potato calico and potato necrosis viruses, 10° or more lower than those for alfalfa yellow mosaic virus. The properties of the last virus are in agreement with those of several of the previously described strains of alfalfa mosaic virus.

An additional difference between alfalfa yellow mosaic and the other viruses in the group is the immune reaction of cucumber, cowpea, pepper, petunia and red clover (Table 1). All the viruses listed except yellow patch and alfalfa yellow mosaic viruses are infectious to cucumber. Only alfalfa yellow mosaic and yellow dot viruses caused systemic infection on

FIG. 1. Symptoms produced by alfalfa yellow mosaic on several hosts: A) Systemic necrotic oak leaf pattern on tobacco. B) Local necrotic lesions on cowpea var. Black Eye. C, D, and G) Local necrotic lesions, systemic yellow blotching, and systemic mottle, respectively, on Pinto bean. E) Yellow primary spots on inoculated leaf of Bountiful bean. F) Local necrotic lesions on Great Northern U.I. No. 123 bean. H) Systemic necrosis on trifoliate leaves of Pinto bean. I) Mottle caused by yellow bean mosaic virus on Pinto bean.

cowpea. Pepper virus was not tested on this species but it is assumed that it is closely related to alfalfa mosaic virus 1. Alfalfa yellow mosaic and yellow dot viruses did not infect pepper, whereas the others did. Although some of the viruses were not tested on petunia and red clover, those that were tested, with the ex-



ception of alfalfa yellow mosaic virus, infected these hosts (Table 1).

Since 1947 there have been 6 reports on new strains of alfalfa mosaic virus, indicating that these viruses are probably being recognized for the first time. In most instances they do not appear to be causing serious damage to the crops they infect. However, the symptoms induced on certain hosts in the greenhouse suggest that they could seriously reduce yields if environmental conditions were favorable. This apparently is true, to a certain extent, of the strain causing tuber necrosis in potato. Oswald (7) reported in 1946 that this virus caused a serious disease of the White Rose variety of potato in fields near Stockton, California, where it usually occurred in the margins of potato fields adjacent to alfalfa plantings. Recently, severe virus disease damage to Ladino clover plantings in California, caused by a virus complex in which one of the alfalfa viruses was involved, was reported (4).

The first alfalfa viruses reported produced only local lesions on inoculated leaves of beans, which would be of no consequence in the field. The more recent strains, however, which produce systemic necrosis and mottling of infected plants could, if widespread, cause considerable damage to commercial bean acreages as do some of the other viruses of bean.

TECHNICAL DESCRIPTION.—*Marmor medicaginis* H. var. **flavovarians** n. var. Common name: Alfalfa yellow mosaic. Hosts: LEGUMINOSAE—*Medicago sativa* L., alfalfa. Experimentally also transmissible to many species of plants including BALSAMINACEAE—*Impatiens holstii*, balsalm; COMPOSITAE—*Zinnia elegans*, zinnia; LEGUMINOSAE—*Phaseolus vulgaris*, bean, *Glycine max*, soybean *Melilotus alba*, white sweetclover, *Pisum sativum*, pea; SOLANACEAE—*Nicotiana tabacum*, tobacco.

Geographical Distribution: Idaho.

Induced disease: Necrotic lesions produced on beans atypical of those produced by the type strain of alfalfa mosaic virus. In some varieties of beans, stem and petiole necrosis followed by severe mottle and necrosis of trifoliate leaves. In other varieties yellow chlorotic local spots on inoculated leaves followed by mottling of these and later formed leaves. In tobacco and *Nicotiana glutinosa* small necrotic local lesions on inoculated leaves and mild mottling of older leaves. In cowpea, reddish-brown necrotic lesions followed by intense yellowing and mottle of later produced leaves.

Transmission: By inoculation of expressed juice in dilution up to 4000 ×.

Thermal Inactivation and Longevity: At 65°-70° in 10 minutes. Resists aging at 18° for 72 hours.

Immunological relationships: Protects beans from infection by Ladino-clover yellow patch and yellow dot viruses.

SUMMARY

A new strain of alfalfa mosaic virus producing both necrotic local lesions and systemic necrosis and mottling on beans was isolated from naturally infected alfalfa in Idaho. On naturally infected alfalfa it produced a bright yellow leaf mottling. It is described, identified, and compared with other strains of the virus. The disease is designated as alfalfa yellow mosaic and it is proposed that the virus be known as *Marmor medicaginis* H. var. **flavovarians** n. var.

Of 24 bean varieties inoculated with alfalfa yellow mosaic virus, all were susceptible, but not to the same degree. In all cases the virus was systemic, but on some varieties necrotic local lesions and systemic necrosis were not produced.

In general, the host range of the alfalfa yellow mosaic virus is similar to that of the other strains of alfalfa mosaic virus. However, certain hosts such as cucumber, cowpea, pepper, petunia and red clover are immune to this virus.

The physical properties of the virus conform with those of several of the previously reported alfalfa mosaic viruses. The virus was inactivated at 65°-70° C. in 10 minutes and in 96 hours at 18°, and withstood a dilution of 4000 ×.

Cross protection studies suggest that alfalfa yellow mosaic, yellow patch and yellow dot viruses are related.

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LITERATURE CITED

1. BERKELEY, G. H. 1947. A strain of the alfalfa mosaic virus on pepper in Ontario. Phytopathology **37**: 781-789.
2. BLACK, L. M. AND W. C. PRICE. 1940. The relationship between viruses of potato calico and alfalfa mosaic. Phytopathology **30**: 444-447.
3. GROGAN, R. G. AND J. C. WALKER. 1948. A pod-distorting strain of the yellow mosaic virus of bean. Jour. Agr. Res. [U. S.] **77**: 301-314.
4. HOUSTON, B. R. AND J. W. OSWALD. 1951. Mosaic diseases of Ladino clover in California. (Abs.) Phytopathology **41**: 940.
5. KREITLOW, K. W. AND W. C. PRICE. 1949. A new virus disease of Ladino clover. Phytopathology **39**: 517-528.
6. MCWHORTER, F. P. 1949. Alfalfa virus N. (Abs.) Phytopathology **39**: 861.
7. OSWALD, J. W. 1950. A strain of the alfalfa-mosaic virus causing vine and tuber necrosis in potato. Phytopathology **40**: 973-999.
8. PIERCE, W. H. 1934. Viroses of the bean. Phytopathology **24**: 87-115.
9. THOMAS, H. R. 1951. Yellow dot, a virus disease of beans. Phytopathology **41**: 967-974.
10. THOMAS, H. R. AND W. J. ZAUMEYER. 1950. Red node, a virus disease of beans. Phytopathology **40**: 832-846.
11. ZAUMEYER, W. J. 1938. A streak disease of peas and its relation to several strains of alfalfa mosaic virus. Jour. Agr. Res. [U. S.] **56**: 747-772.
12. ZAUMEYER, W. J. 1952. Another strain of alfalfa mosaic virus systemically infectious on beans. (Abs.) Phytopathology **42**: 344
13. ZAUMEYER, W. J. AND H. R. THOMAS. 1950. Yellow stipple, a virus disease of bean. Phytopathology **40**: 847-859.

THE CHEMOTHERAPEUTIC ACTIVITY OF BENZOTHIAZOLE AND RELATED COMPOUNDS FOR FUSARIUM WILT OF TOMATO [1]

A. E. Dimond and David Davis [2]

The chemotherapeutic activity of sodium and potassium 2-benzothiazolylthioglycolates was recently noted when they were applied in aqueous solution to the growing medium for control of a number of plant diseases (3). In laboratory and greenhouse experiments, accumulated evidence has shown that these compounds are not active as systemic fungicides; instead they alter the metabolism of the host, thus producing a plant more resistant to disease. Recent studies have indicated an unexpected and highly specific relation of chemical structure to chemotherapeutic activity. Additional experimental results in support of this thesis are reported here.

In assays involving chemotherapeutic potency, tomato plants of standard age were watered with stated concentrations of the test compound for 10 days, after which plants were inoculated with *Fusarium oxysporum* f. *lycopersici*. When the disease was well developed in check plants, a grade for severity of disease was obtained, based on the distribution of vascular discoloration in the stem of each plant. The mean grade over all plants receiving a given treatment, relative to that in corresponding checks, is a measure of the disease control. When a compound is applied to plants in a series of concentrations, the resulting indices of disease severity may be used to construct a concentration-response curve on a logarithmic-probability grid. From this curve is derived an estimate of the concentration which reduces the severity of disease to half that in check plants, the ED 50, and also of the rate at which disease is reduced as concentration is increased (4). This procedure is similar to that used in estimates of fungitoxicity.

Where estimates of fungitoxicity to *Stemphylium* and *Sclerotinia* were made in the present study, the standard spore germination method (5) was used. In other experiments involving an estimate of the fungitoxicity of compounds to *Fusarium*, the test compound was incorporated into liquid nutrient in stated concentrations, and the growth relative to that in check flasks was noted.

In repeated chemotherapy assays, the sodium and potassium salts of 2-benzothiazolylthioglycolate at 125 p.p.m. reduced the severity of disease to 0.0, 5.3, 0.0, and 5.0 per cent of the levels in corresponding check plants. The maximum concentration tolerated by plants without serious injury was 250 p.p.m. To determine whether these compounds were acting in the plant as fungicides, the fungitoxicity to *Stemphylium* and *Sclerotinia* spores was estimated. In these experiments, the concentration to reduce germination significantly was of the order of 10,000 p.p.m. for either compound. Three conclusions are possible: the compound may be (a) vastly more toxic to *Fusarium* than to these 2 fungi, (b) accumulated considerably by the plant or (c) acting by a nonfungitoxic mechanism.

To determine whether or not these compounds are fungitoxic at the levels at which they occur in plants, a series of plants was treated at the maximum nonphytotoxic concentration for the usual period and then macerated in a Waring blendor. The macerated tomato tissue was then slightly diluted with water, and mixed with spores of *Stemphylium* or *Sclerotinia*. Germination of spores was not inhibited.

As a check on the toxicity of these compounds to *Fusarium* itself, the growth of mycelium was followed in Richards' solution containing either compound at concentrations ranging from 25–1250 p.p.m. In flasks containing up to 1250 p.p.m. of these compounds, growth of mycelium was retarded but not stopped. This concentration was about 5 times that which could be applied to the plant without injuring it. Therefore accumulation of a fungitoxic concentration by the plant seems unlikely, since phytotoxicity did not occur. Moreover the inhibition of mycelial growth which this concentration brought about was not sufficient to account for the high reduction in severity of disease recorded in the chemotherapy assays. Accordingly, the chemotherapeutic activity cannot be ascribed to fungicidal action. There remains the possibility that these materials exert their chemotherapeutic effect by altering the resistance of the host. Davis and Dimond (2) reported that these compounds produce slight formative effects upon tomato plants, do not affect reducing sugar levels, and markedly increase the levels of several water-soluble nitrogen fractions in the plant.

The potency of potassium and sodium benzothiazolylthioglycolates as chemotherapeutants led the authors to investigate the chemotherapeutic activity of a number of related compounds. These compounds were applied in a dosage series; dosage response curves were constructed for each compound and, from these curves, values for the ED 50 and slope were estimated (Table 1). All compounds possessing activity had dosage-response curves of the same slope.

Evidently the activity of the potassium and sodium salts of 2-benzothiazolylthioglycolates is the same, and these compounds are the most potent of those studied. Strikingly enough, neither thiazole nor mercaptobenzothiazole shows any activity as a chemotherapeutant, but both benzothiazole and potassium 2-thiazolylthioglycolate are moderately active. Also potassium β-(2-benzothiazolyl) thiopropionate is evidently less active than are derivatives of thioglycolic acid.

The evidence points to the inverse relationship be-

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[2] Plant Pathologist, Crop Protection Institute.

TABLE 1.—*Potency of 2-benzothiazolylthioglycolates and related compounds as chemotherapeutants for Fusarium wilt of tomato*

Compound	ED 50 micromolar
Thiazole	∞
Benzothiazole	130
2-Mercaptobenzothiazole	∞
Potassium 2-thiazolylthioglycolate [a]	220
Sodium 2-benzothiazolylthioglycolate [a]	56
Potassium 2-benzothiazolylthioglycolate [a]	57
Potassium α-(2-benzothiazolyl) thiopropionate [a]	270
Potassium β-(2-benzothiazolyl) thiopropionate [a]	580

[a] Supplied by Rohm & Haas Co., Washington Square, Philadelphia, Pa.

tween fungitoxicity and chemotherapeutic potency of benzothiazole and mercaptobenzothiazole. Table 1 reports mercaptobenzothiazole to be inactive chemotherapeutically, while benzothiazole is moderately active. Horsfall and Rich (6) have noted that benzothiazole shows no fungitoxicity to *Stemphylium* or *Sclerotinia* at 130 μg./cm.^{2} whereas mercaptobenzothiazole has an LD 50 (fungitoxicity) value of about 8.4 μg./cm.^{2}.

Among these compounds the correlation between chemotherapeutic activity and production of a formative effect on tomato plants is very high. Moreover, all chemotherapeutically active compounds produced formative effects which were indistinguishable from one another.

Careful scrutiny of the structure of these compounds leads to the suggestion that the chemotherapeutic activity and formative activity are centered in the thiazole ring. Thus, both benzothiazole and potassium 2-thiazolylthioglycolate are active. Apparently thiazole itself does not readily enter the plant, or else the activity of the nucleus is enhanced when either a benzene ring or an aliphatic side chain with sulfur bridge in the 2 position is added to the thiazole ring.

The diminished activity of potassium β-(2-benzothiazolyl) thiopropionate relative to that of the thioglycolates is of more than passing interest. The formative effects of sodium and potassium 2-benzothiazolylthioglycolates have already been noted (2). In structure these compounds resemble the phenoxyacetic acids; they differ in 2 respects: 1) the presence of a heterocyclic ring instead of a benzene ring and 2) the presence of a thioether linkage to acetic acid instead of an ether linkage. Thimann (7) has called attention to the ability of S and other heteroatoms to replace carbon in growth hormones. It is possible that sulfur behaves analogously to oxygen in contributing activity to synthetic compounds. There is a very high correlation (1) between chemotherapeutic potency among other compounds and their formative activity as exemplified by the synthetic growth hormones.

The aryloxyaliphatic acids are high in hormonal activity when the aliphatic side chain contains an even number of carbon atoms in a straight chain but such activity is reduced when the chain has an odd number of carbon atoms (8). Presumably this effect arises from an actual β oxidation of the aliphatic chain, resulting eventually in a phenoxyacetic acid (active) or in phenol (inactive). Thus 2, 4-dichlorophenoxyacetic acid is highly active, whereas the corresponding propionic acid is much less so. The relative activity of potassium 2-benzothiazolylthioglycolate and of potassium β-(2-benzothiazolyl) thiopropionate as growth regulants and as chemotherapeutants is believed to be strictly analogous, a 2-membered side chain possessing higher biological activity than a 3-membered one. Potassium α-(benzothiazolyl) thiopropionate, with a 2-membered side chain, is again more active than the corresponding β-thiopropionate as a chemotherapeutant. If this reasoning is correct, the lack of potency of mercaptobenzothiazole is analogous in this series of compounds to the lack of potency of the corresponding phenols in the phenoxy series. Apparently a reduced sulfur group in the 2 position renders the molecule inactive.

SUMMARY

The potassium and sodium salts of 2-benzothiazolylthioglycolic acid are very low in fungitoxicity but rather high in chemotherapeutic potency. The evidence indicates that these compounds are not accumulated in the plant to a sufficient degree to account for their chemotherapeutic potency as systemic fungicides.

The chemotherapeutic potency of a number of compounds related to potassium 2-benzothiazolylthioglycolate varies markedly, thiazole and mercaptobenzothiazole having no activity, benzothiazole and potassium α-(2-benzothiazolyl) thiopropionate being moderately active, and sodium and potassium 2-benzothiazolylthioglycolates being highly active. Activity of these compounds as chemotherapeutants is directly related to their formative activity upon plants, and the analogy between them and the phenoxy acids is stressed.

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LITERATURE CITED

1. DAVIS, D. 1952. Inducing disease resistance with plant growth regulators. (Abs.) Phytopathology **42**: 465.
2. DAVIS, D. AND A. E. DIMOND. 1952. Altering resistance to disease with synthetic organic chemicals. Phytopathology **42**: 563-567.
3. DIMOND, A. E. AND D. DAVIS. 1952. 2-Carboxymethylmercaptobenzothiazole salts as chemotherapeutants for plant diseases. (Abs.) Phytopathology **42**: 7.
4. DIMOND, A. E., D. DAVIS, R. A. CHAPMAN, AND E. M. STODDARD. 1952. Plant chemotherapy as evaluated by the Fusarium wilt assay on tomatoes. Conn. Agr. Exp. Sta. Bul. 557, 82 p.
5. HORSFALL, J. G. 1945. Fungicides and their action. Chronica Botanica, Waltham, Mass. 239 p.
6. HORSFALL, J. G. AND S. RICH. 1951. Fungitoxicity of heterocyclic nitrogen compounds. Contrib. Boyce Thompson Inst. **16**: 313-347.
7. THIMANN, K. V. 1951. The synthetic auxins: relation between structure and activity. In F. SKOOG, ed. Plant growth substances. Univ. Wis. Press, Madison, Wis. p. 21-36.
8. WAIN, R. L. 1949. Chemical aspects of plant growth-regulating activity. Ann. Appl. Biol. **36**: 558-562.

A NEW NECROTIC-LESION-PRODUCING STRAIN OF YELLOW BEAN MOSAIC [1]

W. J. Zaumeyer and H. H. Fisher

A new strain of yellow bean mosaic virus was isolated from malformed bean pods collected in eastern Washington in 1950. The infected pods were similar in appearance to those infected with the pod-distorting virus reported by Grogan and Walker (2). Besides producing necrotic local lesions on a number of bean varieties, very unlike those produced by the X strain of yellow bean mosaic virus from gladiolus (6)[2] (Fig. 1, J) or by the pod-distorting strain of yellow bean mosaic, it also produces mottle symptoms more intense on some varieties than those produced by the type strain of yellow bean mosaic virus reported by Pierce (8) and Zaumeyer and Wade (10). This paper gives the results of a comparative study of this new virus strain and others in the yellow mosaic group.

METHODS AND MATERIALS.—All the studies were conducted in greenhouses in which the temperatures were kept between 20 and 27° C. Carborundum powder No. 320 was used as an abrasive in the transfers.

A culture of the type strain of yellow bean mosaic virus from infected bean plants collected in Oregon was secured from F. P. McWhorter. The symptoms produced by the new virus on beans appeared to be similar to those produced by the type strain (Fig. 1, F). The viruses of common bean mosaic and the strain herein referred to as New York 15 mosaic[3] (9) used in the protection tests have been in use at Beltsville for a number of years. The viruses of yellow bean mosaic and New York 15 mosaic were maintained on Pinto U. I. No. 72[4] bean and common bean mosaic virus on Stringless Green Refugee. When not in use, the last 2, being seedborne, were maintained in infected seed.

SYMPTOMS.—The necrotic local lesions of the new strain appear 6-8 days after inoculation. This is a few days longer than is usually required for local lesion production by most other bean viruses that produce such lesions on beans. There are 2 types of necrotic lesions produced, depending on the variety: 1) a distinct and somewhat circular spot (Fig. 1, E), and 2) spreading veinal necrotic lesions which frequently cover a large portion of the leaf (Fig. 1, A and B). When first formed, the distinct circular spots appear similar to those produced by pod mottle and southern bean mosaic viruses (11, 12). Later they turn dark red, are solid, and increase to 2-3 mm. in diameter. The veinal necrotic lesions are stippled and frequently form an intricate pattern of reddish irregular lines and circles often over much of the leaf (Fig. 1, A). The leaf area bounded by the necrotic lines and circles is lighter green than the rest of the leaf. On the underside of the leaves considerable vein and veinlet necrosis is noted. The necrotic lesions later coalesce and form papery scald-like areas followed by defoliation.

The systemic mottle symptoms are rather similar to those produced by the type yellow mosaic virus, but on some varieties as Pinto U. I. No. 72 and Stringless Green Refugee, they are more intense (Fig. 1, C and I). Drooping of the leaf and leaflets at the pulvini, a symptom commonly observed on plants infected with the typical yellow bean mosaic virus, was not produced by the new strain. On varieties such as Michelite, Robust, Small White, and several others the symptoms are similar to those produced by common bean mosaic and New York 15 mosaic viruses. Necrosis of the trifoliate leaves is produced on a few varieties. Stem, petiole, and top necrosis, as are produced by the pod-distorting virus and the X virus on certain varieties, are seldom produced by the new virus strain. Infected plants of many varieties are stunted. No pod malformation has been noted on infected plants in the greenhouse.

INOCULATIONS OF BEAN VARIETIES.—Forty-four varieties of beans were inoculated with the new virus. All were susceptible and fell into 3 classes: 1) susceptible to necrotic local and systemic-mottle infection, 2) susceptible to necrotic local infection alone, and 3) susceptible to systemic-mottle infection alone.

The varieties that belonged in the first category were Black Creaseback, Blue Lake No. 65, Blue Lake Stringless No. 228, Blue Lake, Great Northern U. I. No. 15, Ideal Market, Kentucky Wonder, Michelite, Pinto U. I. No. 72, Potomac, Red Mexican U. I. No. 3, Robust, Small White, and White Half Runner. On all except Kentucky Wonder, Michelite, and Robust the mottle symptoms were fairly severe and in addition some of the trifoliate leaves showed necrosis. On Blue Lake Stringless No. 228 there were stem necrosis and killing of the terminal growing point. Most of these varieties were inoculated with the type strain of yellow bean mosaic virus for comparison. The systemic-mottle symptoms produced by the new virus were more severe than those produced by the type strain, except on Kentucky Wonder, Michelite, and Robust, on which both viruses produced relatively mild infection, and on Small White, which was severely infected by both viruses.

The varieties that were susceptible to local lesion infection only were Great Northern U. I. Nos. 16, 31, and 123. The last-named variety was the only one infected by the type strain of yellow bean mosaic virus, showing a very mild mottle.

The varieties susceptible to systemic mottle infection only by the new strain were Stringless Black Valentine, Blue Lake Stringless No. 231, Bountiful, Cherokee, Contender, Dwarf Horticultural, Florida Belle, Full Measure, Kentucky Wonder White, Logan,

[1] Accepted for publication October 11, 1952.

[2] Unpublished data of the senior writer.

[3] This virus was designated as the Burkholder strain of common bean mosaic by Grogan and Walker (1).

[4] The initials "U. I." indicate that the strain of bean was originated by the University of Idaho.

Low Champion, Landreth Stringless Green Pod, Pencil Pod Black Wax, Plentiful, Puregold Wax, Idaho Refugee, Stringless Green Refugee, Sensation Refugee 1066, U. S. 5 Refugee, Ranger, Rival, Supergreen, Tendergreen, Tenderpod, Topcrop, Unrivalled Wax, and Wade. The symptoms varied from a mild to a severe mottle depending on the variety. Although a few varieties showed a little veinal necrosis on the trifoliate leaves, only Rival showed severe trifoliate leaf necrosis. All the varieties inoculated were susceptible to the type strain of yellow bean mosaic virus, but the symptoms on many differed from those caused by the new virus strain. For example, Stringless Black Valentine, Full Measure, Pencil Pod Black Wax, Puregold Wax, Idaho Refugee, Tendergreen, and Unrivalled Wax were severely mottled and stunted by the new virus but showed mild to moderate symptoms when infected with the type yellow mosaic virus. In contrast, Florida Belle, Blue Lake Stringless No. 231, Dwarf Horticultural and Logan were severely infected with the type virus strain and showed mild symptoms of the new virus. Kentucky Wonder White was eventually killed by the type yellow mosaic virus but showed only mild mottle when infected with the new virus. In general, the Blue Lake varieties were more severely infected by the type strain of yellow bean mosaic virus than by the new virus.

INOCULATIONS OF OTHER SPECIES.—Besides beans, other species were inoculated with the necrotic strain of virus. The ones that were susceptible were white sweet clover, *Melilotus alba* Desr.; lima bean, *Phaseolus lunatus* L. var. Henderson Bush; tepary bean, *P. acutifolius* Gray var. *latifolius* Freeman; mung bean, *P. aureus* Roxb.; broad bean, *Vicia faba* L.; asparagus bean, *Vigna sesquipedalis* (L.) Fruwirth; cowpea, *V. sinensis* (Torner) Savi vars. Chinese Red and Black Eye; and soybean, *Glycine max* (L.) Merrill var. Patoka. On sweet clover the virus produced a mottle. On inoculated leaves of Henderson Bush lima bean it produced white necrotic oak leaf and ring patterns (Fig. 1, H and K). On tepary bean yellow primary spots that appeared on the inoculated leaves were followed by a mottling of the upper leaves and in some cases necrotic spots were produced. On mung bean ringed necrotic local lesions about 1 mm. in diameter formed (Fig. 1, G) and on broad bean solid brownish red local and systemic necrotic lesions appeared (Fig. 1, D). On asparagus bean and Chinese Red and Black Eye cowpeas small necrotic local lesions were observed, followed by a mottle of the upper leaves. On soybean, leaf mottle was produced.

No infection was obtained on the following species: hyacinth bean, *Dolichos lablab* L.; white lupine, *Lupinus albus* L.; alfalfa, *Medicago sativa* L.; adzuki bean, *Phaseolus angularis* (Willd.) W. F. Wight; rice bean, *P. calcaratus* Roxb.; scarlet runner bean, *P. coccineus* L.; urd bean, *P. mungo* L.; pea, *Pisum sativum* L., including the varieties Thomas Laxton, Little Marvel, Early Perfectah, Wisconsin Perfection, Perfected Wales, Asgrow Early Perfection and Rogers Perfection; crimson clover, *Trifolium incarnatum* L.; red clover, *Trifolium pratense* L.; and white clover, *T. repens* L.

Inoculations were also made with the new virus to tobacco, *Nicotiana tabacum* L.; *N. glutinosa* L.; *N. rustica* L.; and cucumber, *Cucumis sativus* L. White local rings approximately 8 mm. in diameter were produced on the inoculated tobacco leaves and large, yellow local rings on the leaves of *N. rustica*. The virus was recovered in both cases. No infection was noted on *N. glutinosa* or cucumber.

PROPERTIES.—The physical properties of the new virus are comparable to those of the type yellow bean mosaic virus. The former was inactivated between 60° and 62° C. after heating for 10 minutes; its dilution end point was between 1-2,000 and 1-4,000, and it lost its infectivity *in vitro* after 32 hours at 18° C.

SEED TRANSMISSION.—Two hundred and twenty-five seeds were harvested from infected plants and planted in the greenhouse. No infection was noted in the plants grown from these seeds.

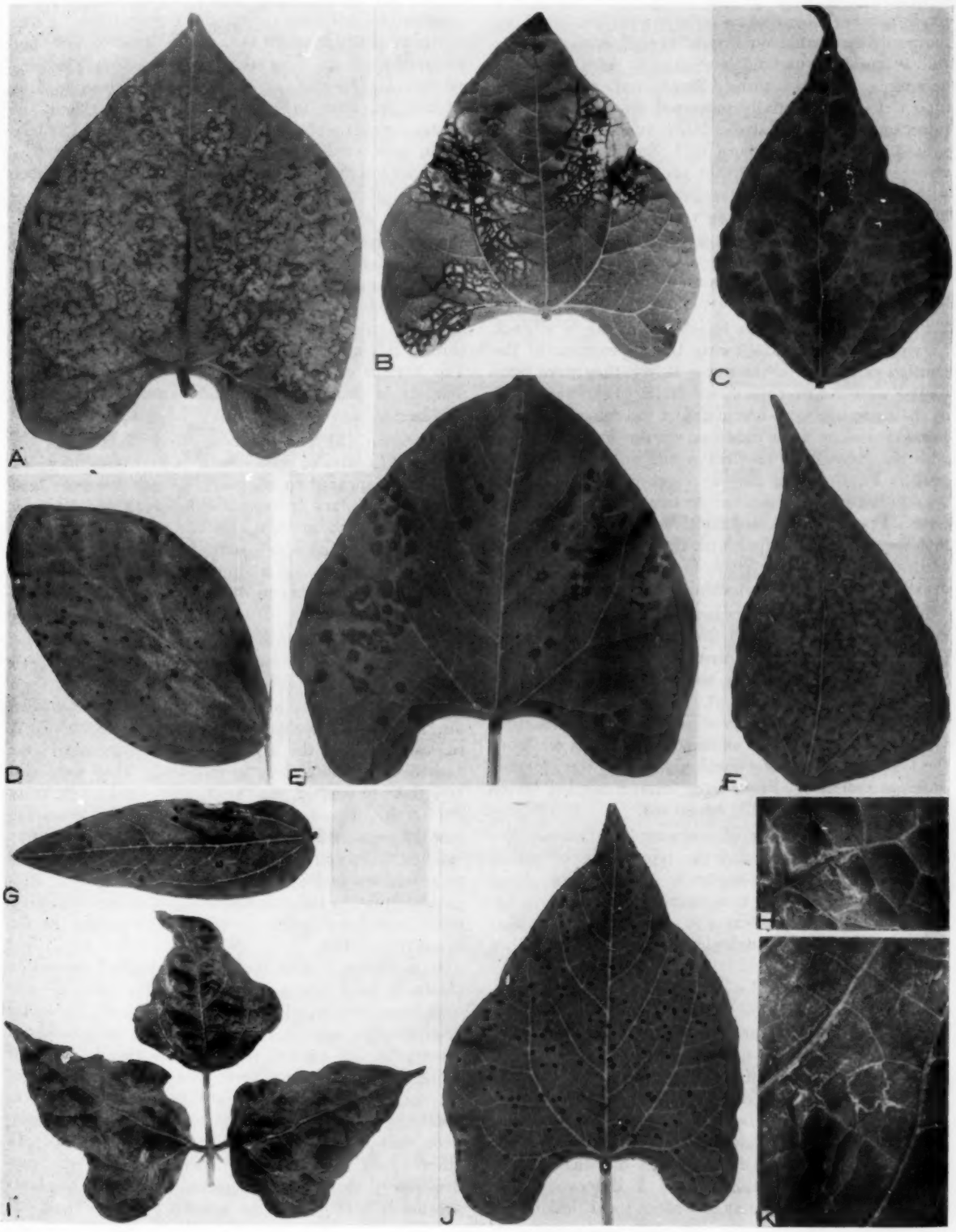
CROSS PROTECTION.—Grogan and Walker (1) showed that infection with common bean mosaic virus effectively immunized bean plants against infection with type yellow bean mosaic virus and that yellow bean mosaic virus partially or entirely immunized bean plants against common bean mosaic virus. Their results were based on suppression of systemic development of the virus on plants inoculated after they were infected by another related virus. Later Grogan and Walker (2) showed that plants infected with the type yellow bean virus and the New York 15 mosaic virus showed a marked reduction in the amount of top necrosis produced when they were inoculated with the pod-distorting virus. Common bean mosaic virus completely inhibited the development of the pod-distorting virus in the Stringless Green Refugee variety.

Since the new virus strain produces necrotic local lesions on beans whereas certain of the other bean viruses do not, cross protection studies could readily be made to determine possible relationships between the new strain and the type strain of yellow bean mosaic virus, common bean mosaic virus, and New York 15 mosaic virus.

Pinto U. I. No. 72, which is resistant to common bean mosaic virus but susceptible to the type yellow

FIG. 1.—Symptoms produced by the necrotic-lesion, X, and type strains of yellow bean mosaic virus on several hosts. A) B) and E) Necrotic local lesions produced by the necrotic strain on Pinto, Great Northern beans U. I. No. 15 and U. I. No. 123, respectively. C) and I) Mottle symptoms produced by the necrotic strain on trifoliate leaves of Pinto bean. D) Necrotic local lesions produced by necrotic strain on broad bean. F) Mottle symptoms produced by type strain on Stringless Green Refugee. G) Local lesions produced by necrotic strain on mung bean. H) and K) Oak leaf and ring patterns produced by necrotic strain on Henderson Bush lima bean. J) Necrotic local lesions produced by the X strain on Blue Lake bean.

mosaic virus and the necrotic virus strain, was inoculated with the type virus strain. About 10 days after the mottle symptoms of this virus appeared, infected leaves were inoculated with the necrotic virus strain and only an occasional necrotic local lesion was produced on the inoculated leaves, whereas on leaves of



previously healthy plants of the same age, abundant lesions were produced. The necrotic virus strain was recovered from later formed mottled leaves of the plants first inoculated with the type yellow mosaic virus and later with the new virus. The fact that a small number of primary lesions were produced by the new virus strain on leaves infected with the type yellow mosaic virus, together with the recovery of the new virus from later formed leaves, indicated that the type virus only partially protected the plants against infection by the new strain. The experiment was repeated with Great Northern U. I. No. 15, which has a virus reaction similar to that of Pinto U. I. No. 72, with approximately the same results. On the mottled leaves infected with the type yellow mosaic virus only a few primary lesions were produced by the necrotic virus strain most of which were on a single plant. The new virus, however, was recovered from the mottled leaves of all the inoculated plants.

Pinto U. I. No. 72 was infected with the New York 15 virus. Ten days following the appearance of the mottled symptoms on the upper leaves, they were inoculated with the necrotic virus strain. Healthy checks of the same age were inoculated at the same time. No primary lesions were produced on the leaves infected with the New York 15 virus, while on the previously healthy Pinto checks they were produced abundantly, thus indicating complete protection by New York 15 virus. From later formed mottled leaves of the plants that were inoculated first with the New York 15 virus and next with the new virus, the latter was recovered, proving again that only partial immunization resulted.

Idaho Pinto, a variety susceptible to common bean mosaic, was infected with this virus and the mottled leaves were inoculated with the necrotic virus strain. Results were comparable to those obtained in the yellow bean virus experiment. Only a few local lesions of the new virus were produced on the leaves previously infected by the common mosaic virus while on the leaves of the healthy check plants they were formed in large numbers. From later formed mottled leaves the new virus was readily recovered.

Although the viruses of common bean mosaic, the New York 15 mosaic, and the type strain of yellow bean mosaic did not completely protect bean plants from infection by the new necrotic strain, it is believed that partial protection is sufficient evidence that the new virus is immunologically related to the other three bean viruses.

Discussion.—From the standpoint of systemic symptoms on beans, lack of seed transmission, physical properties, and cross-protection, it appears that the virus described here is a strain of yellow bean mosaic virus. Several strains of this virus have been described (2, 3, 4, 6, 7,). This necrotic strain is distinct from any others that have been reported in that its host range within the Leguminosae differs from that of the other strains and that it is the first one that has infected species outside this family. It differs from the type strain by producing necrotic local lesions on beans, infecting *Phaseolus lunatus*, *Vigna sinensis* and *V. sesquipedalis* and not infecting *Pisum sativum*, *Trifolium incarnatum*, and *T. pratense*. It is unlike the pod-distorting virus in that it seldom produces stem and top necrosis of beans, which are typical symptoms produced by this virus on a number of varieties. Great Northern 123 is resistant to the pod-distorting virus but susceptible to the new virus strain, which produces necrotic local lesions on it. The pod-distorting virus infects *Trifolium incarnatum* and some varieties of *Pisum sativum* while the new virus does not. The new strain infects *Phaseolus lunatus*, *Vicia faba* and *Glycine max* but the pod-distorting strain does not. Neither virus is infectious to *Trifolium pratense*.

The X strain of yellow mosaic reported by McWhorter and Boyle (6), on Blue Lake beans in Oregon differs widely from the necrotic strain. The X strain virus produces severe stem and tip necrosis and killing of Blue Lake plants; the necrotic strain generally does not. Likewise the X strain is infectious to crimson clover, red clover, and pea; the new strain is not. The X virus causes mottling of broad bean while the new strain produces local and systemic necrosis on this host.

Hagedorn (3) and Hagedorn and Walker (4) described 4 isolates of yellow bean mosaic virus, of which isolate 1 appeared to be fairly distinct from the type strain. It differs from the virus described here by infecting *Pisum sativum*, *Trifolium incarnatum*, and *T. pratense*, all of which were not infected by the necrotic virus strain, and by not infecting *Vigna sinensis*, which was susceptible to the new virus. Isolate 1 has a dilution end point of 1 to 100,000, while the new virus is inactivated at about 1 to 3,000 dilution.

The necrotic virus strain is unlike the 5 yellow bean mosaic isolates reported by McWhorter (7) from Oregon in that these produced mottling in *Vicia faba*, but no necrosis typical of the new strain. Only 2, representative of the area, were tested on red clover and they were infectious to this host. They were also infectious to certain pea varieties as Alaska and Early Perfectah. These results differentiate them from the new strain. Although the necrotic lesion virus was isolated from malformed bean pods, it was impossible to reproduce pod distortion in the greenhouse. It is possible that in the original infected material another virus may have been present, but was lost in the original isolation.

It is believed that the type of partial protection shown in bean plants infected with the virus of common bean mosaic, of New York 15 mosaic, or yellow bean mosaic, against formation of the necrotic local lesions but not against systemic infection by the new virus differs from any heretofore reported. In most cases, viruses producing only systemic infection in plants have protected against related viruses that produce only local necrotic lesions on these plants. In other cases, protection has been shown by the suppression of systemic development of a virus in plants previously infected with a related systemic virus. It

has been demonstrated that some related strains of viruses do not completely protect against others (5). Partial protection, however, is considered as evidence that viruses are immunologically related.

SUMMARY

A new local-necrotic-lesion-producing virus isolated from infected bean pods collected in eastern Washington is described, identified, and compared with other bean viruses. It is believed that it is a new strain of the yellow bean mosaic virus.

Besides producing necrotic local lesions on beans, the virus produces mottle symptoms more intense on some varieties than those produced by the type strain of the yellow mosaic virus. Top necrosis, a typical symptom produced by the pod-distorting-virus and the X virus, strains of the yellow bean mosaic virus, is seldom produced by the new virus.

Of 44 bean varieties tested all were susceptible and fell into 3 classes: varieties susceptible to both necrotic local and systemic mottle infection; varieties susceptible to local infection alone; and varieties susceptible to systemic mottle infection alone.

Phaseolus lunatus, *Vigna sesquipedalis*, and *V. sinensis* are susceptible to the necrotic strain but are resistant to the other strains of yellow bean mosaic virus. *Pisum sativum*, *Trifolium incarnatum*, *T. pratense*, and *T. repens* were not infected by the necrotic virus strain while certain other strains infect these species. The new virus also infected *Nicotiana tabacum* and *N. rustica*. No previously reported strain of the yellow mosaic virus has infected these hosts.

The properties of the new virus are comparable to those of the type strain. The viruses of common bean mosaic, New York 15 mosaic, and the type strain of yellow bean mosaic partially protected against infection by the new virus.

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LITERATURE CITED

1. GROGAN, R. G. AND J. C. WALKER. 1948. Interrelation of bean virus 1 and bean virus 2 as shown by cross-protection tests. Phytopathology **38:** 481-493.
2. GROGAN, R. G. AND J. C. WALKER. 1948. A pod distorting strain of yellow mosaic virus of bean. Jour. Agr. Res. [U. S.] **77:** 301-314.
3. HAGEDORN, D. J. 1951. The reaction of Perfection type peas to Wisconsin bean virus 2 isolated from pea. Phytopathology **41:** 494-498.
4. HAGEDORN, D. J. AND J. C. WALKER. 1950. The relation of bean virus 2 to pea mosaic in Wisconsin. Phytopathology **40:** 684-698.
5. MATHEWS, R. E. F. 1949. Criteria of relationship between plant virus strains. Nature **163:** 175.
6. MCWHORTER, F. P. AND L. BOYLE. 1946. X disease and other forms of yellow bean mosaic in western Oregon. Oreg. Agr. Expt. Sta. Cir. of Infor. No. 379. 6 pp.
7. MCWHORTER, F. P. 1949. Susceptibility of selections of Perfection peas to strains of yellow bean mosaic. U.S.D.A. Plant Dis. Reptr. **33:** 139-144.
8. PIERCE, W. H. 1934. Viroses of the bean. Phytopathology **24:** 87-115.
9. RICHARDS, B. L. AND W. H. BURKHOLDER. 1943. A new mosaic disease of beans. Phytopathology (Note) **33:** 1215-1216.
10. ZAUMEYER, W. J. AND B. L. WADE. 1935. The relationship of certain legume mosaics to bean. Jour. Agr. Res. [U. S.] **51:** 715-749.
11. ZAUMEYER, W. J. AND L. L. HARTER. 1943. Two new virus diseases of bean. Jour. Agr. Res. [U. S.] **67:** 305-327.
12. ZAUMEYER, W. J. AND H. R. THOMAS. 1948. Pod mottle, a virus disease of beans. Jour. Agr. Res. [U. S.] **77:** 81-96.

Relation of Temperature to Internal Cork Development in Stored Sweet Potato Roots.[1] ROBERT AYCOCK,[2] J. S. COOLEY,[3] M. B. HUGHES,[2] AND W. L. SMITH, JR.[3] This paper presents results of a study initiated in 1950 of the effect of storage temperature on internal cork development in sweet potato roots. Nielsen[4] reported that sweet potato roots stored at 78° to 80° F. developed larger and more numerous corky lesions than those from the same lot stored at 55° to 60° for comparable periods. Severity of symptoms was no greater in samples at the lower temperatures than in those at harvest. Wilcox and Ezell,[5] who stored roots at 55°, 60°, and 70°, observed that the highest percentage of roots showed symptoms and that the largest lesions developed at 70°. Results presented here confirm those already reported.

In the present study, Porto Rico sweet potatoes harvested in 1950 from 2 sources, each with a different level of symptom expression, were used. Seventy-eight per cent of the roots from Source 1 showed corky spots prior to bedding. Seed roots of Source 2 were obtained earlier that year from northern Louisiana where internal cork disease had not been reported. Periodic examinations of 515 of these roots failed to reveal internal cork symptoms.

Nine bushels of roots were harvested from plants from each source on November 7, 1950, then graded and distributed as uniformly as possible into 2 sets of 18 half-bushel samples. Three half-bushel samples from each source were examined immediately for internal cork lesions. The remaining baskets were placed in the curing room at Beltsville, Maryland, on November 8. The sweet potatoes were cured at 85° F. and about 85 per cent relative humidity for 10 days. Three half-bushel samples from each source were then stored for approximately 5 months at the test temperatures (50°, 55°, 60°, 70°, and 85°).

The incidence and severity of internal cork were determined in the freshly harvested and stored roots by cutting them transversely into ⅛ to ¼ in. slices. Each slice was rated[6] for severity of symptoms, using a modification of Nusbaum's scale[7] (Fig. 1).

Data obtained from roots from the high-symptom-

TABLE 1.—*Incidence and severity of internal cork in Porto Rico sweet potatoes after storage for approximately 5 months at various temperatures* [a]

Storage temperature (°F.)	Total roots examined (number)	Percentage of roots with lesions		Mean slice rating [b]
		Mean percent	Mean angle [c]	
(At harvest)	196	43.9	41.52	.....
50	185	43.3	41.18	0.06
55	183	45.8	42.62	0.07
60	189	47.9	43.80	0.10
70	188	87.1	68.98	0.87
85	195	88.7	70.39	1.42
L.S.D. (.05)	.....	.....	8.21	0.14
L.S.D. (.01)	.....	.....	11.49	0.19

[a] The assistance of E. J. Koch, Biometrical Services, Plant Industry Station, Beltsville, Md., in the statistical treatment of the data is gratefully acknowledged.

[b] Mean slice ratings obtained at harvest were not included in the statistical analysis.

[c] The percentage data were transformed to angles (angle = arc sin √ percentage) prior to statistical analysis in order to stabilize the variances.

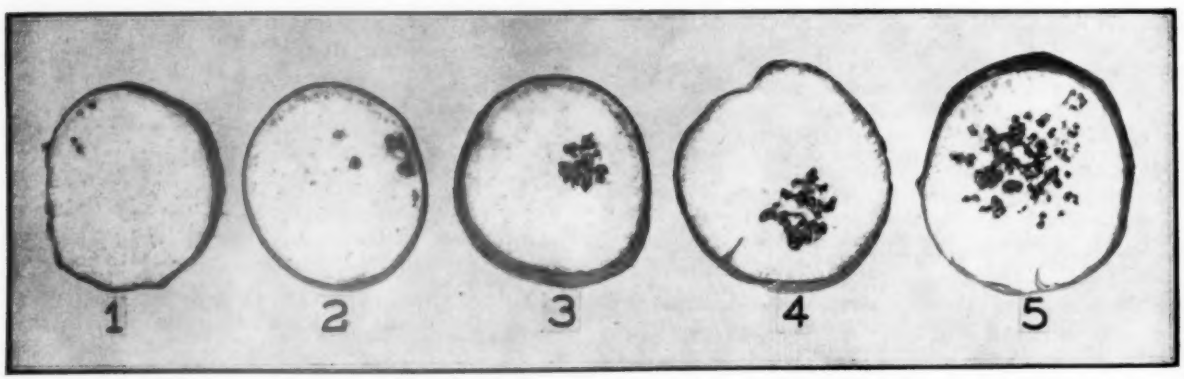


FIG. 1. Classification used in rating incidence and severity of internal cork symptoms. Each classification is determined by the diameter of spots or aggregates of spots. Left to right: 1, less than 1/16 in.; 2, 1/16 to ¼ in.; 3, ¼ to ½ in.; 4, ½ to 1 in.; 5, greater than 1 in.

[1] Approved by the Director as Technical Contribution No. 203 of the South Carolina Agricultural Experiment Station.

[2] Edisto Experiment Station, Branch of Clemson Agricultural College, Blackville, S. C.

[3] Division of Handling, Transportation, and Storage of Horticultural Crops, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Maryland.

[4] Nielsen, L. W. 1951. Influence of temperature on the development of internal-cork symptoms in sweet potato roots. (Abs.) Phytopathology 41: 28.

[5] Wilcox, Marguerite W. and B. D. Ezell. 1951. Storage temperature and the development of internal cork in sweet potato. Phytopathology 41: 477-479.

[6] The writers are indebted to Mrs. H. F. Smart and J. C. Etheredge for assistance in rating the samples.

[7] Nusbaum, C. J. 1950. Internal cork of sweet potatoes. S. C. Agr. Exp. Sta. Bull 381.

level source are in Table 1. A significantly greater percentage of roots developed corky spots at 70° and 85° F. than at the lower temperatures, but the differences in percentage of corky roots stored at 70° and 85° were not significant. The percentage of corky roots at 50°, 55°, and 60° was not significantly greater than that observed in roots at harvest.

The severity of internal cork symptoms as reflected by mean slice rating was also significantly greater at the 2 higher temperatures. More rapid increase in size of lesions at 85° F. resulted in a mean slice rating significantly greater than at 70°. No significant differences were noted in the amount of internal cork at the 3 lower temperatures.

In roots from the low-symptom-level source, the same levels of significance were noted. In roots stored at 50°, 55°, and 60° F. the maximum percentage of corky roots in any one sample was 3 per cent. No corky roots were observed in 2 samples. At a temperature of 85°, however, 15 per cent of the roots showed symptoms of internal cork.

From a control standpoint, the results indicate that storage temperatures of 50° to 60° F. will prevent any appreciable development of symptoms beyond those already present at harvest. Since sweet potatoes may become chilled if stored continuously at 50°,[8] storage at 55° to 60° is recommended.

[8] Cooley, J. S. and Helen F. Smart. 1950. Spoilage of sweet potatoes at 50°, 55° and 60° F. (Abs.) Phytopathology 40: 869-870.

Initial Evidence of the Presence of Tristeza, or Quick Decline, of Citrus in Florida. THEODORE J. GRANT[1] AND HENRY SCHNEIDER.[2] West Indian lime varieties inoculated with the virus of tristeza in Brazil and South Africa and with that of quick decline in California have been reported to have similar veinclearing and stem pitting symptoms.[3, 4, 5, 6] Since West Indian lime seedlings show symptoms much sooner and have more specific symptoms than sweet orange trees on sour orange rootstocks, they are useful as test plants for detection of the presence of tristeza, or quick decline, virus in areas where the disease has not been recognized.

Tests for detecting the possible presence of tristeza in Florida were begun in September 1951 with the inoculation of West Indian lime plants of the Key, Beledy, and Kirk varieties. The bottle graft method was employed for the inoculations. Twigs from trees suffering from unidentified declines were approach-grafted into the stems of healthy test plants. The cut ends of the twigs were immersed in vials of water until the union was completed. By February 1952 4 of the first 70 inoculated plants showed veinclearing symptoms. The budwood had been collected from one tree in a citrus grove about 10 mi. west of Orlando, Florida, and from one tree near Avon Park, about 80 mi. south. Examination of citrus groves at Ocoee and Winter Garden, near Orlando, revealed the presence of a few scattered individual and small groups of sweet orange trees on sour orange rootstock in a state of decline. Twigs from these trees were collected for inoculation of West Indian limes, and bark samples for anatomical study were obtained from the point of union of the sweet orange top and the sour orange rootstock.

The presence of sieve tube necrosis below the bud union of orange trees on sour orange rootstock and reactions occurring secondary to the necrosis have been useful for identifying trees affected by quick decline.[7] When these secondary reactions are intense, the diagnosis is considered positive. When they are less intense, experience has shown that the trees might be declining from bud union disturbances resulting from quick decline virus or from some other unknown cause and the diagnosis must therefore be recorded only as possible quick decline. If sieve tube necrosis is present and the characteristic secondary anatomical changes are absent the results are considered indeterminant. The results are considered negative if the phloem at the bud union is normal. This classification refers to quick decline, but similar anatomical symptoms have been found in trees affected with tristeza.[8]

Results of the present study of 26 bark samples from 26 suspected trees in Florida are as follows: 11 positive, 8 possible, and 7 negative. The anatomical diagnosis of bark samples is a valuable and rapid means of indicating the presence of tristeza, or quick decline. It constitutes, however, only one step in the obtaining of proof of the presence of the virus. Transmission of the virus to healthy tests plants, such as the West Indian limes, with the subsequent development of typical veinclearing and stem pitting symptoms, is important.

Bottle graft inoculations with twigs from a total of

[1] Principal plant pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Orlando, Fla.

[2] Assistant plant pathologist, Citrus Experiment Station, University of California, Riverside, Calif.

[3] Costa, A. S., T. J. Grant and S. Moreira. 1950. A possible relationship between tristeza and the stem pitting disease of grapefruit in Africa. Calif. Citrog. 35: 504, 526-528.

[4] Grant, T. J., A. S. Costa and S. Moreira. 1951. Studies of tristeza disease of citrus in Brazil. V. Further information on the reactions of grapefruits, limes, lemons, and trifoliate hybrids to tristeza. Calif. Citrog. 36: 310-311, 324-26, 328, 329.

[5] McClean, A. P. D. 1950. Virus infections of citrus in South Africa. Farming in South Africa. 25: (293) 261-262; 25: (294) 289-296.

[6] Wallace, J. M. and R. J. Drake. 1951. Newly discovered symptoms of quick decline and related diseases. Citrus Leaves. 31(2): 8-9, 30.

[7] Schneider, Henry, J. M. Wallace, and J. E. Dimitan. 1950. The pathological anatomy of bud union tissues of orange trees and its value in the diagnosis of quick decline. Phytopathology 40: 24.

[8] Schneider, Henry, A. A. Bitancourt, and Victoria Rosetti. 1947. Similarities in the pathological anatomy of quick decline- and tristeza-diseased orange trees. Phytopathology 37: 845.

13 declining field trees resulted in the production of definite veinclearing on the leaves of 26 West Indian lime plants. Limes bottle-grafted with twigs from 4 other field trees produced faint veinclearing on 8 test plants. Examination of 5 of the earliest inoculated lime plants that showed definite veinclearing revealed the presence of pits on the stem beneath the bark. Attempts to transmit the disease from 3 infected West Indian lime test plants to healthy test plants have, to date, resulted in the development of definite veinclearing on 6 test plants.

Observations in the screenhouse of the infected West Indian lime plants with veinclearing on the leaves show that most of the plants have a tendency to produce new growth free of veinclearing. This reaction is in distinct contrast to the experience in Brazil, where on similar lime plant material inoculated with the severe form of tristeza virus, leaf yellowing and cupping and gradual decline of plants followed the initial veinclearing symptoms. The reactions of inoculated lime plants obtained to date in Florida indicate that a mild or intermediate form of the tristeza virus rather than the more severe form is present.[9]

Under field conditions, tristeza, or quick decline, symptoms similar to those described for the disease in other parts of the world, have been observed on the following sweet orange varieties on sour orange rootstocks: Temple, Valencia, Lue Gim Gong, and Parson Brown.

The fact that 11 of the 26 bark samples studied showed phloem reactions typical of those associated with tristeza, or quick decline, plus the production of veinclearing on 26 and stem pitting symptoms on 5 of the inoculated West Indian lime plants, constitutes evidence of the presence of a form of the tristeza, or quick decline, virus in Florida.

[9] Grant, T. J., and A. S. Costa. 1951. A mild strain of the tristeza virus of citrus. Phytopathology 41: 114-122.

REPORT OF THE 44TH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The American Phytopathological Society held its 44th Annual Meeting on September 8, 9, and 10, 1952, at Cornell University, Ithaca, New York, in conjunction with the American Institute of Biological Sciences. The Council met on September 7 and 10; business meetings took place on September 8 and 10. Over 500 members and guests attended. A total of 158 formal papers were presented, consisting of 133 original research, 16 symposium, and 9 Fungicide Colloquium papers. Two joint sessions were held with the Mycological Society of America and one with the Potato Association of America. The symposia dealt with Disease Resistance, Plant Parasitic Nematodes and Physiology of Parasitism. Conferences were held on Oak Wilt, Legume Viruses, Stone Fruit Viruses, the National Tomato Disease Screening Program, and on Extension. Original research papers, presented in 15 sessions, may be classified thus: Diseases of various crops and plants 55, Fungicides including Chemotherapy and Antibiotics 26, Mycology including Fungus Physiology 25, Viruses 18, and Nematology 9.

The annual dinner, held at the Statler Inn, was attended by 385 members and guests, constituting the full capacity of the Auditorium. Entertainment was provided by amateur magician District Attorney Frederick B. Bryant of Ithaca.

Council for 1953:

James G. Dickson, President (1 yr.), Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin

James H. Jensen, Vice President (1 yr.), Department of Botany, North Carolina State College, Raleigh, North Carolina

S. E. A. McCallan, Secretary (3 yrs., expires 1953), Boyce Thompson Institute for Plant Research, Inc., Yonkers 3, New York

Saul Rich, Treasurer of the Society and Business Manager of Phytopathology (3 yrs., expires 1955), Connecticut Agricultural Experiment Station, P. O. Drawer 1106, New Haven 4, Connecticut

W. C. Price, Editor-in-Chief, Phytopathology (3 yrs., expires 1954), University of Pittsburgh, Pittsburgh 13, Pennsylvania

George F. Weber, President Elect, Department of Plant Pathology, University of Florida, Gainesville, Florida

George L. McNew, Retiring President, Boyce Thompson Institute for Plant Research, Inc., Yonkers 3, New York

George A. Zentmyer, Councilor-at-Large (2 yrs., expires 1953), Citrus Experiment Station, Riverside, California

George W. Fischer, Councilor-at-Large (2 yrs., expires 1954), Department of Plant Pathology, State College of Washington, Pullman, Washington

C. E. Cox, Potomac Division, Department of Botany, University of Maryland, College Park, Maryland

E. W. Hanson, North Central Division, Plant Pathology Division, University of Wisconsin, Madison 6, Wisconsin

W. B. Hewitt, Pacific Division, Division of Plant Pathology, University Farm, Davis, California

F. O. Holmes, Northeastern Division, Rockefeller Institute for Medical Research, 66th St. & York Ave., New York 21, N. Y.

Coyt Wilson, Southern Division, Department of Botany and Plant Pathology, Alabama Polytechnic Institute, Auburn, Alabama

Representatives:

A.A.A.S. Council. H. C. Young (1952-1953), P. R.

MILLER (1953-1954).

American Institute of Biological Sciences. J. C. WALKER (4 yrs., expires June 30, 1954).

Agricultural Research Institute, National Research Council. W. F. JEFFERS; E. L. LECLERC, alternate.

Division of Biology and Agriculture, National Research Council. S. J. P. CHILTON.

Food Protection Committee, Food and Nutrition Board, National Research Council. G. L. MCNEW.

Board of Editors, American Journal of Botany A. J. RIKER.

American Type Culture Collection. H. H. MCKINNEY.

Associates Food and Container Institute. H. H. THORNBERRY.

Standing Committees:

Donations and Legacies
FREEMAN WEISS, Chr.; N. J. GIDDINGS, P. P. PIRONE, W. H. TISDALE

Extension
L. O. WEAVER, Chr.; A. H. BAUER, C. F. BISHOP, S. B. FENNE, R. J. HASKELL, J. E. LIVINGSTON, C. E. SCOTT, E. G. SHARVELLE, ARDEN SHERF, J. C. WELLS

Finances and Investments
A. E. DIMOND, Chr.; SAUL RICH (ex-officio), W. H. TISDALE, R. H. WELLMAN

International Cooperation
E. C. STAKMAN, Chr.; A. A. BITANCOURT, K. S. CHESTER, G. H. COONS, CARLOS GARCES O, W. F. HANNA, J. G. HARRAR, LEE LING, H. A. RODENHISER, J. A. STEVENSON, FREEMAN WEISS, F. L. WELLMAN

Phytopathological Classics
C. W. BOOTHROYD, Business Manager; L. C. KNORR, Editor.

Placement
E. E. HONEY, Chr.; S. J. P. CHILTON, M. W. GARDNER, R. J. HASKELL, L. S. HITCHNER, R. S. KIRBY, L. M. MASSEY, H. A. RODENHISER

Public Relations
THOMAS SPROSTON, Chr.; G. H. HEPTING, P. R. MILLER, J. D. MOORE, G. K. PARRIS, ARDEN SHERF, R. A. YOUNG, G. A. ZENTMYER.

Plant Disease Prevention
E. B. LAMBERT, Chr.; S. B. FRACKER, J. G. LEACH, P. R. MILLER, E. C. STAKMAN, RUSSELL STEVENS

Regulatory Work and Foreign Plant Diseases
E. G. REX, *Chr.*; C. R. ORTON, L. O. WEAVER, W. H. WHEELER, R. P. WHITE

Seed and Plant Material Certification
JOHN HARDISON, Chr.; C. H. ARNDT, WAYNE BEVER, REINER BONDE, L. C. COCHRAN, J. T. MIDDLETON, W. H. WHEELER

Special Committees:

Fungicide Colloquium
L. G. UTTER, Chr.; S. I. COHEN, J. W. HEUBERGER, L. S. HITCHNER, R. H. WELLMAN, J. D. WILSON

Fungicide Nomenclature
G. A. ZENTMYER, Chr.; R. H. GRUENHAGEN, J. B. HARRY, CURT LEBEN, R. G. OWENS, D. W. POWELL, E. A. WALKER

Industrial Relations
R. H. WELLMAN, Chr.; C. J. KRISTER, L. G. UTTER, F. L. WINTER, S. G. YOUNKIN

Membership
J. R. SHAY, Chr.; J. J. CHRISTENSEN, W. J. HOOKER, ARTHUR KELMAN, THOR KOMMEDAHL, CURT LEBEN, SAUL RICH (ex-officio), P. M. SIMMONDS, C. E. YARWOOD

Nomenclature and Classification of Plant Viruses
FRANK MCWHORTER, Chr.; C. W. BENNETT, L. C. COCHRAN, K. W. KREITLOW, H. H. MCKINNEY, A. F. ROSS, FREEMAN WEISS

Subcommittee on Virus Type Culture Collection
H. H. MCKINNEY, Chr.; R. W. FULTON, T. J. GRANT

Publication of Special Material
E. L. LECLERC, Chr.; W. H. BRAGONIER, J. J. CHRISTENSEN, G. L. MCNEW

Teaching of Plant Pathology
W. H. BRAGONIER, Chr.; M. F. KERNKAMP, J. G. LEACH, L. D. LEACH, W. C. PADDOCK, J. R. SHAY, THOMAS SPROSTON, H. H. THORNBERRY, G. F. WEBER

Utilization of Plant Pathologists and Facilities in National Emergencies
H. H. THORNBERRY, Chr.; A. L. ANDERSON, H. T. COOK, M. F. KERNKAMP, C. H. KINGSOLVER, J. G. LEACH, C. L. LEFEBVRE, REX THOMAS

Temporary Committees for 1953:

Fungus Nomenclature VIII International Botanical Congress
J. A. STEVENSON, Chr.; J. H. MILLER, C. G. SHAW, W. C. SNYDER

Method of Collecting and Disseminating Data on New Fungicide Tests
L. G. UTTER, Chr.; J. W. HEUBERGER, P. R. MILLER, A. G. NEWHALL, R. H. WELLMAN

Report of the Secretary. On September 1, 1952, the total membership was 1,572, consisting of 131 life members, 7 emeritus members (2 of whom are also life members), 1,354 regular members and 82 applicants. Reports have been received, since the last meeting, of the death of seven members; E. W. Bodine, O. C. Boyd, Mel T. Cook (charter), H. S. Jackson (charter), James Johnson, J. S. Wiant and George J. Wilds.

S. E. A. MCCALLAN, *Secretary.*

Report of the Treasurer. Statement of account for the fiscal period October 1, 1951–June 30, 1952.

Receipts:		
Balance on hand, October 1, 1951	$8,255.48	
Membership dues	1,895.00	
Total Receipts		$10,150.48
Expenditures:		
Office of the Secretary	673.84	
Office of the Treasurer	434.18	
Dues, AIBS	1,000.00	
Expenses, Placement Committee	23.90	
Expenses, Annual Meeting (Cincinnati)	249.27	
Total Expenditures	$2,381.19	
Balance on hand, June 30, 1952	7,769.29	
		$10,150.48

A. E. DIMOND, *Treasurer.*

Report of the Business Manager for *Phytopathology*. Statement of account for the fiscal period October 1, 1951–June 30, 1952.

Receipts:		
Balance on hand, October 1, 1951	$18,002.26	
Subscriptions and Member subscriptions	17,140.66	
Advertisements	1,928.00	
Reprints & Excess Illustrations	3,025.85	
Sale of Back Issues	1,875.80	
Sustaining Associate Memberships	200.00	
Donation, Rockefeller Institute	600.00	
Interest on Investments	333.35	
Investments liquidated	13,562.42	
Total Receipts		$56,668.34
Expenditures:		
Manufacture & Distribution of Phytopathology	9,966.56	
Cost of Reprints	1,412.36	
Office of Business Manager	708.65	
Office of the Editor-in-Chief	2,393.77	

News Letter	5.94	
Office of the Advertising Manager	307.95	
Decennial Index	1,709.44	
Cost of New Investments	13,809.32	
Collector of Internal Revenue	320.33	
Refunds	295.05	
Miscellaneous expenses	119.81	
Purchase of back volumes for resale	50.00	
Total Expenditures	$31,099.18	
Balance on hand, June 30, 1952	25,569.16	
		$56,668.34

A. E. DIMOND, *Business Manager.*

Sinking Fund.

The principal amount of the Sinking Fund was increased during the fiscal year by $99.21 (representing Dr. Horsfall's travel expenses to Cincinnati, paid by the A. P. S. to him as president and donated by him to the Sinking Fund). The principal amount of this fund, accordingly, is $10,962.55 and is invested as follows:

U. S. Savings Bond, Series G	$1,000.00
U. S. Savings Bonds, Series F	1,150.00
Northwestern Federal Savings & Loan Ass'n. (certificate)	2,000.00
Family Finance Corp.—common stock, 7 shares at par	7.00
Westinghouse Electric Co.—common stock, 22 shares at cost	849.08
Standard Oil Co. of Ohio—common stock, 50 shares at cost	2,018.65
American Cyanamid Co.—common stock, 20 shares at cost	2,160.47
Johns-Manville—common stock, 25 shares at cost	1,664.27
On deposit, New Haven Savings Bank	113.08
Total	$10,962.55

Lyman Memorial Fund.

The principal amount of this account was increased from $3,383.43 to $3,405.94 to permit investment of the fund in common stock. Interest accrued in this fund was used to increase the principal amount. It is currently invested as follows:

Westinghouse Electric Co.—common stock, 35 shares at cost	$1,350.81
American Telephone & Telegraph—common stock, 13 shares at cost	2,055.13
Total	$3,405.94

***Phytopathology* Contingency Fund.**

As now set up the Contingency Fund is largely a liquid fund which, in addition to the principal amount, is to receive the dividends and interest derived from the Sinking Fund and the Lyman Memorial Fund. It is invested as follows:

Johns-Manville—common stock, 7 shares at cost	$ 466.00
Jefferson Federal Savings & Loan Ass'n.	669.86
District Bldg. & Loan Ass'n.	2,005.06
Perpetual Bldg. Ass'n.	1,339.86
Arlington Fairfax Bldg. & Loan Ass'n.	1,231.62
Prudential Bldg. Ass'n.	451.12
Arundel Federal Savings & Loan Ass'n.	3,058.70
Total	$9,222.22

A. E. DIMOND, *Business Manager.*

Report of the Auditing Committee for the Fiscal Period, October 1, 1951 to June 30, 1952.

We have examined the books of the Treasurer of The American Phytopathological Society and of the Business Manager of PHYTOPATHOLOGY for the period from October 1, 1951 to June 30, 1952. We found all receipts and expenditures, and all funds of this Society and of PHYTOPATHOLOGY, accurately recorded.

Signed: R. H. WELLMAN.
PAUL H. SCHULDT.

Report of the Business Manager of *Phytopathological Classics* for the fiscal year, October 1, 1951 to September 30, 1952. Number of copies of Phytopathological Classics sold during fiscal year is 63, having a total value of $53.25. Seven copies (No. 2 through No. 8) sent gratis to Ing. K. Minev, Macedonia, Yugoslavia, for library use. Number classics on hand September 30, 1952: No. 1, None; No. 2, 67; No. 3, 145; No. 4, 216; No. 5, 431; No. 6, 495; No. 7, 454; No. 8, 639.

Receipts:			
Cash balance on October 1, 1951		$834.62	
Received during fiscal year 1951-52			
For classics sent out	$38.25		
On accounts due in previous fiscal years	15.00	53.25	$887.87
Disbursements:			
United States postage		$10.00	
Cash balance on September 30, 1952		877.87	$887.87
Due on accounts September 30, 1952			
Present fiscal year	$ .75		
Previous fiscal years	23.50		
Total due on accounts	$24.25		

CARL W. BOOTHROYD, *Business Manager.*

Report of the Advertising Manager. During the first 9 months, 55.25 pages of ads in PHYTOPATHOLOGY carried 72 advertisements. Advertisers paid $2,438.30. The income to the Society after deduction of agency commissions and cash discounts to advertisers was $2,192.13. Seventeen and ½ pages are under contract for 23 advertisements in the last 3 months. The estimated gross income from these will be $770.40, and the income after deductions of agency commissions and cash discounts is expected to be $688.71. The gross income for the 12 months is estimated to be $3,208.70, and the income after deductions is expected to be $2,880.84.

Estimated expenses of advertising manager including telegraph and postage, $20.00, clerical assistance, $72.00, commission, $50.00 (5 per cent of $1,000.00) and $188.08 (10 per cent of $1,880.84), total $330.08. The total income to the Society will be approximately $2,550.76.

LEONARD J. ALEXANDER, *Advertising Manager.*

Report of the Editor-in-Chief. When the present Editor-in-Chief succeeded Dr. Helen Hart in January 1952, make-up for the January and February issues of PHYTOPATHOLOGY had already been prepared and additional manuscripts were ready for press. The switch in editorship thus was gradual and, under the expert guidance of Dr. Hart, was made with a minimum of confusion.

Change to the 2-column format came also with the January 1952 issue. As a result of the latter change more space than heretofore available has been provided, due mostly to use of a larger size page and smaller size type, and partly due to better possibilities for utilization of space in arranging tables and illustrations. A page in the present format contains as much, or more, printed matter as 1.9 pages in the former single-column format. Thus the 520 pages in the first 9 issues of volume 42 of PHYTOPATHOLOGY are equivalent to 988 pages, approximately 10⅓ issues, of volume 41. This increase in amount of printed matter, combined with authors' cooperation in condensing papers, has made possible continued prompt publication of papers in PHYTOPATHOLOGY.

During the year, PHYTOPATHOLOGY adopted the method of citing literature recommended by the AIBS Publications Committee, of which Dr. A. J. Riker is chairman. In the August issue, literature is cited according to the new method in some papers and according to the old in others, depending upon when the manuscripts were sent to press.

In the first 9 issues of volume 42, 184 authors published 109 articles (415¼ pages), 23 notes (22 pages), and 3 obituaries (3 pages). Also, in the first 9 issues, 468 abstracts (71¼ pages) were published, including 175 (26 pages) for the 1951 annual meeting, 145 (22 pages) for the 1952 annual meeting, 17 (3 pages) for the Northeastern Division, 44 (6¼ pages) for the Southern Division, 25 (4¼ pages) for the Potomac Division, and 62 (9½ pages) for the Pacific Division. The remaining 8½ pages were taken up with 2 official notices (1 page) and the annual report of the Society (7½ pages).

On August 15, 10 manuscripts were in the hands of the reviewers, 12 in process of being edited after approval by reviewers, 7 returned to authors for revision, 19 in press, and 11 in galley proof. Between November 15, 1951 and August 15, 1952, 21 manuscripts were withdrawn or rejected. PHYTOPATHOLOGY has no appreciable backlog of manuscripts at the moment; papers are being published as rapidly as they can be got ready and into print.

The Editor-in-Chief is pleased to acknowledge the financial assistance of The Rockefeller Institute for Medical Research and the Sustaining Associates, the excellent service rendered by Mr. John Ferguson 3d and associates of Monumental Printing Company, the advice of the Editors and Associate Editors, the cooperation of the Business Manager and other officers of the Society, and the editorial assistance of Mr. and Mrs. Wilson S. Borland.

W. C. PRICE, *Editor-in-Chief.*

Report of the Editor of News. During 1952, "News" was published in each number of the Journal on the page facing the inside back cover. News was also listed in the Table of Contents as a definite part of the Journal. The Editor feel that these 2 improvements may have played a part in stimulating the increased flow of news items that have reached his desk. There still are items of phytopathological interest, however, that escape to other journals before they come to our attention but it is hoped that as "News" becomes more firmly established these items will be sent to the news editor of The American Phytopathological Society as well as to news editors of other journals. The short distance between the publishing house in Baltimore and the Editor's office at Beltsville enabled the Editor to delay submitting final copy until shortly before publication date. This has permitted inclusion of more timely items.

K. W. KREITLOW, *Editor.*

Report of Representative on the Governing Board of the American Institute of Biological Sciences. AIBS has continued to develop soundly and conservatively. The 2 annual meetings (Columbus and Minneapolis) have been successful both scientifically and financially. The 1953 meeting has been definitely set for Madison in early September. Projected future dates are Gainsville, 1954, East Lansing, 1955, New England, 1956, Pacific Coast, 1957. The AIBS Bulletin has continued to be published at a high standard of form and content and also is largely self-supporting. AIBS has cooperated with AAAS and NEA in exploring the future relations of scientists to manpower shortages both at college and pre-college levels. This coincides with the awakening interest of many societies in providing vocational guidance material at the secondary school level which is informative of the needs and opportunities in the biological sciences. The National Science Register contract was continued in cooperation with the Office of Scientific Personnel, NRC. About 63,000 questionnaires have been sent to biologists of which about 21,000 have been returned. During the year 4 issues of Bio-Sciences Newsletter were prepared. Of this some 1,000,000 copies were distributed by the State Department after translation into several foreign languages. The budget approved for 1952-53 totaled $57,950 of which $12,000 comes from society dues, the remainder being derived from advertising, convention receipts, Rockefeller grant, and dues of associates and affiliates.

J. C. WALKER.

Report of the Representative to the Division of Biology and Agriculture, National Research Council. The Division held its annual meeting in Washington on May 2. A mimeographed report of the activities of the Division was distributed. The Division has continued its support of several activities of special interest to the APS, most of which were enumerated in the Annual Report for 1951. Committees on Plant Crop Ecology and on Developmental Biology have recently been activated .

Members of the Council responded promptly to a request for suggestions of activities of interest to the Society that might be brought to the attention of the Division. The several proposals received will be discussed in the near future with the Secretary of the Division.

CURTIS MAY.

Report of Representative to the Food Protection Committee, Food and Nutrition Board, National Research Council. The Society's representative attended all meetings of the Food Protection Committee and its Subcommitte on Pesticides during the past year. The committee released a pamphlet on "Use of Chemical Additives in Foods," the original draft of which was drawn up by the Society's representative. Two printings of 10,000 copies each have been exhausted and the article has been reprinted verbatim in 6 different journals so the public has become acquainted with the real nature of the problem of chemical additives and the necessity for use of fungicides and other pesticides.

The committee's Subcommittee on Pesticides, under the chairmanship of Dr. George C. Decker, has been working on minimum standards of performance and safety required for new pesticides. Through the good offices of the subcommittee, members of industry and federal regulatory bodies have been encouraged to consolidate their viewpoints and set up what appears to be a fairly workable outline to guide inventors in evaluating promising new chemicals.

The Food Protection Committee will continue its activity during 1952, so the Society should appoint a liaison representative to represent its interests.

GEORGE L. MCNEW.

Report of Representative on Board of Editors, American Journal of Botany. *The American Journal of Botany* is back on publication schedule. Manuscripts are commonly printed within 6 months after they are received. Abstracts are no longer published. This is the first year it has charged $25 per page after the 8-page free limit. The journal is in a good financial condition.

A. J. RIKER.

Report of the Committee on Donations and Legacies. The Committee is of the opinion that definite objectives should be set by the Council for which support is needed and can best be derived from the income from invested funds. With specific and formally approved goals in view, the prospect of obtaining contributions from the Society membership and from other sources would be materially improved.

The Committee offers the following suggestions as objectives to be supported by the Society from the income from invested funds, but does not assume as its appropriate function the complete formulation of such objectives:

1) Providing a limited number of gratuitous subscriptions to PHYTOPATHOLOGY for the benefit of individuals or

institutions in countries that are at a serious disadvantage in dollar-exchange.

2) Giving financial assistance to Biological Abstracts to expedite the publication of its annual indexes.

3) Giving financial assistance to the American Type Culture Collection for the publication of its catalogs of cultures of microorganisms of plant viruses.

4) Subsidizing the publication in PHYTOPATHOLOGY of meritorious research articles of greater than average length, and of papers submitted by non-members of the Society when it would be a hardship to assess the entire cost against the individual.

With these and perhaps other definite objectives in view, the Committee proposes to undertake in 1953 a general solicitation of the Society membership for contributions to a $10,000 sustaining fund which, together with the present sinking fund and other investments, will provide sufficient income to support these projects.

FREEMAN A. WEISS, *Chairman.*

Report of the Extension Committee. Seventeen extension pathologists were in attendance at the annual meeting at Cincinnati. As announced in the program of the 43rd annual meeting, 2 extension sessions were held. A Joint Extension Symposium with the American Association of Economic Entomologists was well attended. Papers presented on proposed legislation were timely. The speakers were: W. W. Sunderland, Chairman National Agricultural Chemicals Association, and E. L. LeClerg, Agricultural Research Administration, U. S. D. A. Three speakers discussed "Methods of Stimulating Insect and Disease Control Practices."

Several commercial interests acted as hosts at the extension plant pathologists' luncheon. Afterwards, 2 papers were presented on the subject of "All-Purpose Sprays and Dusts for Home Orchards and Gardens." Dr. Ellsworth Wheeler presented the formulae for tree fruits as recommended for Massachusetts and Mr. C. F. Bishop gave an excellent account of the work on garden vegetable insect and disease control in West Virginia.

L. O. WEAVER, *Chairman.*

Report of the Committee on Finances and Investments. During the past 3 years, the Finance Committee, following the recommendation of the Council, has reviewed the investments of the Society with the purpose of reinvesting 50 per cent of its funds in common stocks of sound and growing enterprises. As pointed out in the 1951 report of the Finance Committee, a program was decided upon and some of the sales and purchases made. The program was completed early in the current year.

Results of this investment program are promising, as shown in the Report of the Treasurer of the Society and Business Manager of PHYTOPATHOLOGY. It is realized, however, that dividends from common stocks are not as certain as those from guaranteed bonds. Diligence must be exercised to be sure that a sound financial position is maintained.

W. H. TISDALE, *Chairman.*

Report of the Placement Committee. During 1952, 53 plant pathologists have had their applications in the active files of the Placement Committee. Prospective employers requested applications in an attempt to fill 24 positions. Sixty-four applications have been sent to 23 prospective employers.

Records show that 2 plant pathologists have obtained positions to date during 1952 through the efforts of the Committee.

E. E. HONEY, *Chairman.*

Report of the Committee on Public Relations. A general survey of public phytopathology news was attempted by the committee during the past year. The survey was not so complete as it could have been. Committee members neglected to clip stories and news items, and only 3 members of the committee made a serious attempt to gather the necessary information. However, from reports, correspondence, clippings, magazine stories, University and Experiment Extension releases, some conclusions can be made.

The news coverage on plant pathology is more extensive than was at first realized. The greatest coverage is by the daily press. Daily newspapers have the largest reading population (4 readers for every paper sold). Most of the reports were short news articles and were of the so-called popular type. Many were realized by the national news services, such as AP, INS, and UP. Some stories from New England had extensive United States coverage. Several of these stories, reported to the committee, appeared in all sections of the country. One story with a California date line (AP service) even appeared in the largest paper in Vermont.

A noteworthy impression was the failure of news releases from the various extension services to get national coverage. Such stories nevertheless get local coverage. News coverage of all types by local newspapers seems to be adequate and is constantly bringing plant pathology to the attention of the public.

There is a definite lack of coverage in the large magazines. This can be overcome by better and more appealing stories, and more people will have to submit stories.

Feature stories on Plant Pathology as a science or the sciences in Plant Pathology are decidedly lacking in all public relations releases. The secret of this difficult situation is the lack of good writers within the Society.

The committee recommends a change in its personnel. Fewer members, interested in public relations, who can write feature stories on science in an appealing manner should be effective. A diligent search should be made within the Society for such young active members. Any suggestions will be very much appreciated.

THOMAS SPROSTON, *Chairman.*

Report of the Committee on Plant Disease Prevention. The Committee on Plant Disease Prevention has had no formal meeting since the last annual meeting of the Society, but individual members of the committee have been actively working, in their official and unofficial capacities, for the desired goal. It appears now that implementation of the committee's recommendations will depend largely upon the interest and support that we can get from the Civil Defense Administration. The committee, through its contacts, is keeping before the Civil Defense Administration the importance and necessity of adequate means of plant disease prevention in national defense. The committee will do all in its power to see that plant disease prevention receives proper recognition when and if the Civil Defense Administration receives adequate financial support from Congress.

J. G. LEACH, *Chairman.*

Report of Subcommittee of Plant Disease Prevention Committee on Seed and Plant Material Certification. The Subcommittee has restricted its efforts to obtaining data to ascertain whether or not provisions as prescribed by the International Crop Improvement Association are sufficient to minimize losses from seed-carried pathogens. A preliminary survey of opinions of plant pathologists throughout the United States has indicated a need for more specific consideration of diseases and an increase in stringency of tolerance, especially in foundation and registered stocks. For example, 1.0, 1.0, 2.0 and 0, 1.0, 2.0 per cent tolerance is allowed in foundation, registered, and certified stocks for bean blight and bean mosaic, respectively. Pathologists have generally indicated that these diseases increase so rapidly under favorable conditions that tolerance should be 0 in both foundation and registered stocks and near 0 in certified stocks. Pathologists further recommend

that where effective seed treatments are known they should be required for production of foundation and registered seed stocks, and that other economic seed-borne diseases should be added to those already listed by the I.C.I.A. as soon as measures usable in certification procedures are available.

The Subcommittee hopes to continue its survey and to issue a full report for publication in a suitable journal. It wishes to express its appreciation for the cooperation received from the Pathological Standards Committee of the International Crop Improvement Association, and hopes to offer them substantial material assistance.

JOHN R. HARDISON, *Chairman.*

Report of the Committee on Resolutions. Be it resolved: that The American Phytopathological Society at its 44th annual meeting does hereby express its sincere appreciation:

1. To Dr. T. C. Byerly, Dr. C. J. Hylander, Dr. S. L. Meyer and associates of the American Institute of Biological Sciences for their efforts in arranging and coordinating the meetings of the various affiliated societies, including the A.P.S.

2. To President D. W. Malott, the Trustees of Cornell University and Dean W. I. Myers of the College of Agriculture for use of their excellent facilities.

3. To Dr. C. E. F. Guterman and the members of his local committee for their efforts in arranging for the various sessions.

4. To Dr. George Lawrence of Cornell University, Mrs. Ileen Stewart of the AIBS, and their staffs for cooperation in providing news coverage of Society affairs.

5. To Dr. E. E. Clayton, Dr. A. C. Tarjan and Dr. J. P. Hollis for arranging the three Special Symposia which appeared on the program.

6. To friends in Industry, whose names appear on the annual Banquet Program, for their contributions toward the success of the Annual Banquet.

7. To Mr. J. W. Conner and the Statler Inn for their contribution to the success of the Annual Banquet.

8. To District Attorney Fred B. Bryant for excellent entertainment at the Annual Banquet.

9. To the retiring officers and Council members for their progressive and sound administration of the affairs of the Society since the last Annual meeting.

GEORGE F. WEBER
CARROLL E. COX

Elections and Appointments. A committee was appointed by the Council to count the ballots for the election of new officers. The following were declared elected: President Elect, George F. Weber; Vice President, James H. Jensen; Councilor-at-Large, George W. Fischer.

The Council recommended and the Society approved the appointment for 1953-1955 of Saul Rich as Treasurer and Business Manager of PHYTOPATHOLOGY, and of C. S. Holton as an Editor and M. T. Hilborn, W. J. Hooker, W. F. Jeffers and E. O. Olson as Associate Editors of PHYTOPATHOLOGY.

Representatives, Standing Committees, Special Committees and Temporary Committees were approved and appointed as listed above.

Interim and Ad Hoc Appointments. President McNew designated B. H. Davis representative to the inauguration of President Lewis W. Jones at Rutgers University, New Brunswick, New Jersey, May 8, 1952, and Lawrence P. Miller as delegate to the Centennial Ceremonial Session of the American Pharmaceutical Association, Philadelphia, Pennsylvania, August 20, 1952. A committee consisting of W. C. Paddock, chairman, K. W. Kreitlow, P. R. Miller and H. A. Rodenhiser was designated to investigate the possible use of plant pathologists in the Point IV program.

The Society approved the following recommendations by the Council:

1. Accepted the reports of Officers, Representatives, Standing Committees and Special and Temporary Committees.

2. Elevated the Subcommittee on Regulatory Work and Foreign Plant Diseases and the Subcommittee on Seed and Plant Material Certification, respectively, of the Standing Committee on Plant Disease Prevention to full Standing Committee status.

3. Elevated the Temporary Committee on Industrial Relations to a Special Committee.

4. Discontinued the Special Committee on Sustaining Associates, the Special Committee on Testing and Results of Newer Fungicides and the Interim Committee on the Point IV Program and thanked them for their fine efforts.

5. Discharged the Temporary Committee on a New Name and thanked Chairman Horsfall and his associates for their commendable but fruitless efforts to find a suitable common name for the Society.

6. Appointed a Temporary Committee on Method of Collecting and Disseminating Data on New Fungicide Tests.

7. Appointed a Temporary Committee on Fungus Nomenclature to advise and represent the Society at the VIII International Botanical Congress, Paris, 1954.

8. Instructed the Secretary to thank the Committee on Publication of Special Material for their study on the costs of publishing a history of plant pathology. Also to call to their attention the volume on the life of Erwin F. Smith, sponsored by the American Philosophical Society and the manuscript being prepared by F. V. Rand on the biography of L. R. Jones and to recommend tabling their program until the above manuscripts can be examined. Further suggested that the Committee consider a search for good manuscripts suitably illustrated that could be fittingly used in a monograph series to be published by the Society.

9. Instructed the Secretary to advise the Divisional Secretaries that, in advance of their Divisional Meetings, they request the Editor-in-Chief of PHYTOPATHOLOGY to appoint a special review committee of 3 members to examine the Divisional abstracts of papers preparatory to acceptance for publication in PHYTOPATHOLOGY.

10. Accepted an invitation to Class B membership in The Agricultural Research Institute sponsored by the National Research Council.

11. Ammended the Constitution and By Laws to provide for a Student Membership in the Society as announced in PHYTOPATHOLOGY **42**: 366. 1952. (Students wishing to qualify for Student Membership must apply to the Treasurer on appropriate forms for this membership which begins in 1953).

12. Amended the Constitution regarding the selection of officers and councilors after having been duly notified of the proposal 1 month in advance as follows: Article V—Selection of Officers and Councilors. Section 1. (Phytopathology **37**: 265. 1947). For third last sentence reading —"If the same person should receive sufficient ballots to qualify for nomination to more than one office, his name shall be placed on the final ballot as a candidate for only the higher office, as listed in Article III, Section 1"—substitute—"If the same person should receive sufficient ballots to qualify for nomination to more than one office his name shall be placed on the final ballot as a candidate for only the office for which he receives the greater number of ballots, and in the case of an equal number of ballots he shall be entered as a candidate for only the higher office as listed in Article III, Section 1."

13. Authorized the publication of a new membership list to be prepared by the Secretary's office and published as Section 2 of a regular issue of PHYTOPATHOLOGY.

14. Authorized the publication in PHYTOPATHOLOGY of such symposium papers as are comprehensive in nature and meet the editorial requirements of a special review committee to be composed of a chairman designated by the Editor-in-Chief from among the Editors and additional

members selected by the chairman. Such reprints are to be available to the authors without extra cost.

15. Authorized the publication of obituaries in PHYTOPATHOLOGY with the exemption of the special reprint charge.

16. Authorized the appropriation of $850.00 for the use of Phytopathological Classics to accelerate and expand their program.

17. Authorized the Special Committee on Teaching of Plant Pathology to prepare a suitable brochure for students on careers in Plant Pathology which is to be submitted to the Council for acceptance, whereupon the Council will consider means of dissemination.

18. Deferred action on the request to help support the American Type Culture Collection pending receipt of a more definite program. The Society, however, signifies its willingness to support in part any reasonable program on fungi or viruses that would promote plant pathology.

19. Accepted into Annual Membership all 82 applicants.

The following resolutions were endorsed by the Society:

1. BE IT RESOLVED that The American Phytopathological Society takes this opportunity to express its appreciation and hearty commendation to Dr. Clarence J. Hylander, Executive Secretary of the American Institute of Biological Sciences, for his devotion and energetic execution of duties in behalf of the Institute during this trying period of its organization and establishment. Through his efforts a sound organization has been created which should promote the welfare and cause of biology in the field of plant pathology and in allied fields.

2. BE IT RESOLVED that The American Phytopathological Society encourages its members to cooperate on a voluntary basis with the Bureau of Entomology and Plant Quarantine to prevent the promiscuous introduction of living cultures of plant pathogens, to notify quarantine officials of any proposed introduction, and to follow the general procedures established by the Bureau of Plant Industry, Soils and Agricultural Engineering for its own staff concerning such introductions.

3. BE IT RESOLVED that the Society accepts with gratitude the report of Chairman E. C. Stakman of the Standing Committee on International Cooperation and expresses its appreciation for the initiative and effective efforts he has exercised in interesting the authorities of UNESCO and foreign countries in the problems of plant disease control and need of world-wide plant disease reporting service.

4. BE IT RESOLVED that The American Phytopathological Society expresses its gratification that the Food and Agriculture Organization of the United Nations is undertaking a World Reporting Service for Plant Diseases and Insect Pests. The American Phytopathological Society will encourage and assist this project to the limit of its ability.

5. BE IT RESOLVED that The American Phytopathological Society, cognizant of the importance of the plant disease problems of Latin America and cognizant of the significant contributions that the Rockefeller Foundation is making toward their solution, hereby expresses its gratification that the Foundation has in operation a program of basic and applied research in plant pathology in several countries, that the Foundation has provided opportunities for international meetings and conferences of Latin American phytopathologists, and that the Foundation is continuing its long view program for the training in plant pathology of outstanding young scientists.

The American Phytopathological Society appreciates the progress already made and commends the Foundation for its effective efforts.

Interim Action by the New Council: The new Council with President J. G. Dickson met on September 10 and took the following action:

1. Appointed W. C. Paddock a delegate to the VIII International Botanical Congress in Paris, July, 1954.

2. Agreed to hold the 1954 Annual Meeting in Denver, Colorado, during late August or early September, and appointed J. A. Pinckard, Jr., Chairman of the Local Arrangements Committee.

S. E. A. MCCALLAN, *Secretary*

CAPTAN—A COINED NAME FOR THE FUNGICIDAL CHEMICAL N-TRICHLOROMETHYLTHIO TETRAHYDROPHTHALIMIDE

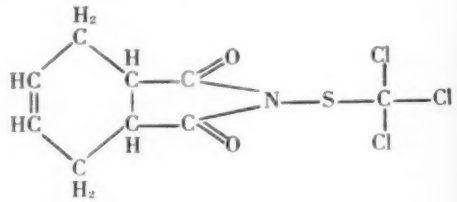
The word "*Captan*" has been selected as a coined name for the fungicidal chemical N-trichloromethylthio tetrahydrophthalimide and approved September 25, 1952 by the Interdepartmental Committee on Pest Control. The name has also been approved by the American Phytopathological Society, and the American Chemical Society is agreeable to its use. *Captan* formerly was designated as SR-406 and ORTHOCIDE.

Captan is in the process of being registered by California Spray-Chemical Corporation as a trade-mark in the United States and certain foreign countries. The California Spray-Chemical Corporation has taken steps to transfer the name to the supplementary list in the Trade-Mark Division from the list where it originally was registered for application as a trade-mark. The name *captan* therefore is available for use as a common name for designating this chemical.

The name "*captan*" applies to the pure chemical and a technical grade shall indicate the percentage of captan present.

Empirical formula: $C_9H_8O_2NSCl_3$

Structural formula:



—Interdepartmental Committee on Pest Control, H. L. Haller, Chairman, S. W. Simmons, Secretary.